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THE DEVELOPMENT OF THE SCHULTZE DUPLICATION FROM THE EGG OF RANA FUSCA PARTS I-IV

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PART I. INTRODUCTION

The monozygotic brown frog twins produced by O. Schultze in 1894 have been severely neglected in development physiology research. Four authors have reported on repetitions of Schultze's experiment, but only G. Wetzel (1895) was successful in determining more exact information concerning the development of this duplication. He confined himself, however, to the investigation of only one form of twins, which, as already indicated in Schultze's data, may develop in a large variety of ways. Since 1895, nothing significant has been published on the subject. This could lead to the conclusion that the problem has been solved theoretically. It has been determined from experimentation and problematics of the last decade that the origin of the Schultze duplications has great significance in the development physiology of the amphibian embryo and, for this reason, it deserves renewed investigation.

At first we wished to confine ourselves to a very narrow range of questions, but we were then led into a thorough study of the development processes which occur in the frog egg, when it is rotated and held compressed by plates in a forced position. The embryogenesis of these eggs depart from the norm to such an extent that the principles of this phenomena and its manifold variations may only be fully understood through a very thorough examination. Our results contain a number of gaps, including several which may be filled without much difficulty. We feel that these gaps may be filled at a later date, since we believe that the results already achieved illuminate and further the solution to the problem of the causality of primitive development in amphibians from a standpoint which has received little attention in the past. The experiments were conducted in the years 1924-1926 and a few of the facts and the conclusions to be drawn therefrom were published in two intermediary reports (Schleip and Penners, 1925 and 1926). The present study will be supplemented by the results of experiments conducted by Miss Charlotte Wittmann.

A. Historical

The following is a summary of the state of knowledge on the Schultze duplication which was prevalent before this study was undertaken.

O. Schultze /308

More than 30 years ago, O. Schultze (1894-) attempted to prove that the normal influence of gravity is absolutely necessary for the organic development of animals. He did this with a simple method employing frog eggs. Unexpectedly, his experiments with the eggs produced twins in the form of 2 individual frogs grown together. Each of the frogs was biologically more or less complete.

Rana fusca eggs were pressed between two glass plates immediately after fertilization. This was done in such a manner that the egg was placed on a glass plate with the light pole upward and fertilized. Thin glass strips were glued on the ends of the glass plates. These strips were just thick enough to cause sufficient compression on the eggs when another glass plate was placed on top of them. The plates were kept in position by two rubber bands and were then laid in water in a saucer which stood exactly horizontally, so that the eggs were oriented normally, i.e., with the dark egg pole upward. During the stage from the beginning to the completion of the first cleavage, Schultze rotated the plates by 180°, so that the light, vegetal pole was upwards and the eggs were forced to continue development in this position. It is known that frog eggs orient themselves within their cortex so that the light, heavier pole is downward and the darker, lighter pole is upward. The occurrence of this self-orientation was hindered by the captive position of the eggs between the glass plates.

The eggs remained in the press for about 20 hours at room temperature. Then, still before the beginning of gastrulation the pressure was released; the eggs remaining in the inverted position. The result of this procedure was that Schultze obtained a large number of more or less complete monozygotic twins. In one experimental series for example, 50 percent of the treated eggs yielded twins, most of which were Duplicitas anterior, some were ventral twins, and others were, in the terminology of Spemann (1918), Duplicitas cruciata. In one single case, the twins were so oriented to one another that the cephalic end of one lay in the direction of the caudal end of the other.

One other result obtained by Schultze should be mentioned. It was shown that eggs, which started their development in normal position (just as the twins in the press), but were first rotated so that the light, vegetal pole faced upward in the 8-cell stage, were soon characterized by a typical coloration of the light field. After this, the eggs changed into an equal cleavage type. The start and further course of gastrulation is always abnormal in such embryos. "When a round plug is formed, it is always surrounded by abnormally puffy lips; in other cases the blastopore is an irregular elliptical or star-shaped slit with a correspondingly formed yolk plug." (1894a, pages 126-127). These embryos die at the end of the formation of the abnormal embryonic

medullary tube and "a swimming larva never develops from an equally segmented egg" (loc. cit., page 127).

It naturally occurs that pressed and rotated eggs are not held absolutely in this forced position and do not remain in a position with /309
their vegetal field upwards. Many eggs, which have not been sufficiently pressed return partially or completely to their original positions. The smaller the degree of return, the greater are the anomalies which occur. According to Schultze, one may achieve all possible degrees of deformities with this method, ranging from deformities of individual organs to the greatest distortions of organic formation, in which "the degree of deformity has a direct ratio to the size of the angle between the direction of gravity and the axis of the abnormally oriented egg." (1894b, page 9).

These experiments led Schultze to the conclusion that the normal effects of gravity are absolutely necessary for normal embryonic development, a conclusion which has no interest for us in this study and which has been shown to be untenable by many other authors. On the other hand, some other details furnished by Schultze concerning the duplication are of interest to us.

He obtained duplications only if the eggs were pressed and held with the heavy pole facing upwards between the beginning and end of the 2-cell stage. "Duplications never occur, if the egg is rotated into an abnormal position before the 2-cell stage or when the egg has split into four, eight or more cells." (1894b, page 10). Schultze also explains that a major condition for duplication is that the pressing must be truly complete, and that the egg cannot turn back in the slightest degree. The light pole must remain exactly in an upward facing position. If the eggs rotate even slightly, this results not in double embryos, but rather in the most various deformities of another type.

If the pressure and rotation are successful, a very characteristic coloration of the upward facing light field soon occurs. The heavier light-colored yolk sinks and the more lightly pigmented substance rises. In typical cases, one can usually determine after one hour that the light vegetal field on top begins to shrink on two sides perpendicular to the direction of the first plane of cleavage. This process progresses slowly until, at the end of cleavage, a thin, light streak may be seen on the blastula above the vegetal field and exactly in the direction of the first cleavage. During the entire course of cleavage, this streak on the blastula shows the exact location of the first cleavage and continues to show it during the first stages of gastrulation. It is therefore an excellent orientation mark. In one series, Schultze found this light streak to be pointed in a direction perpendicular to the first plane of cleavage. He assumed that this exception to the rule represented a frontal position of the first cleavage, which, according to Roux, would

represent the appearance of the real first cleavage during the second splitting stage (anachronism). This light-colored streak may also be lacking but when it does occur, the gastrulation forms always show a typical deviation from the norm.

The rearrangement of the substance of the egg is also indicated by the fact that the cells during the cleavage stages in the vegetal surface have become smaller than the animal cells.

A linear, deep cleavage forms exactly in the direction of the light streak, and therefore, except in the unusual cases mentioned, also in the direction of the first level of division. This cleavage forms the blastopore. In the blastopore area, the white coloration of the light streak gradually disappears in that this material is tucked in and is /310
drawn into the split. This split may also form when no light streak is present and it occurs in various lengths. It can extend from the equator to the vegetal pole, from the animal to the vegetal pole, or it may cover the entire vegetal surface. It can also cleave the animal end to form an approximately normal blastopore. Finally the split can pass around the whole embryo and divide it into two halves. Such a very noticeable gastrulation phenomenon may cause the egg to split by a deep cleft into two more or less sharply separated halves. Each part receives the matter from one of the two half-blastomeres.

Depending on the type of duplication, the embryonic medullary ridges are formed in various ways. In the Duplicitas anterior, which occurs quite often, on only one end of the gastrulation groove on the vegetal surface there occurs a fork-shaped crack. Since this crack directly becomes the embryonic medullary groove, Schultze found this to be the explanation for the formation of the two-headed duplication. In ventral twins, medullary ridges and grooves form on each half of the gastrula and are not related to the gastrulation groove. They lie adjacent on the vegetal surface at first, but gradually are displaced so that the two embryos are fused at the abdomen in the area of the first plane of cleavage. Each embryo is formed therefore from one of the two half-blastomeres.

The third characteristic form, the Duplicitas cruciata, is formed when the gastrulation split, which encircles the entire embryo in the direction of the first cleavage, often transforms its edges into medullary ridges along its whole length, whereby it itself becomes the medullary groove. The ridges to the right of the gastrulation groove form a common head anlage at one point, just as do those lying to the left of the gastrulation groove. "In this case, each half-blastomere yields two half-embryos, which do not fuse with one another, but rather fuse alternately in the first plane of cleavage (see Figure 1a).

The formation of duplications from the bifurcated, rotated egg was explained by Schultze—who was in agreement with the ideas of O. Hertwig—

by the fact that the rotation process causes the egg's center of gravity to be lost. Furthermore, each half-blastomere attempts to recover its molecular equilibrium by forming a new center of gravity and isolating itself from its sister cell. This destroys normal reciprocal influence exerted by the two sister cells on one another. The division of the egg's substance into two independent individualities appears to Schultze to be a necessary condition for the formation of a duplication. Schultze believed that his experiments proved that, in the case mentioned, each half-blastomere formed a whole embryo. Literature on the subject evaluates the Schultze experiment from this standpoint.

Roux

Even before Schultze expressed his opinion on the cause of the formation of duplications (1894c), Roux attempted to explain this phenomenon on the basis of the shift of substances, which had been determined in rotated eggs by von Born (1885). This explanation took place during the discussion of Schultze's lecture at the Anatomical Congress. Roux immediately thereafter (1895, pages 597-598) was able to be more precise /311
in his explanation, which consisted of the fact that gravity caused rearrangement of the egg's substance as a result of inversion. Each half-blastomere obtains an arrangement of plasma substances equivalent to those of the whole normal egg. The intermediate position of nutritive yolk in the area of the first plane of cleavage provides for the independent further development of the two cells.

It should also be noted that Roux had conducted such an experiment earlier, which was reported in the paper mentioned above (1895). He did not, however, produce any duplications in these experiments. A repetition of his experiment did not produce any duplications: 45 Rana esculenta eggs were held in a fixed position by light dampening of the periblast and rotated immediately after the occurrence of the first cleavage. The negative results of his experiments led Roux to the conclusion that in addition to inversion of the divided frog eggs, there must be strong flattening caused by compression in order that each half-blastomere may attain the necessary arrangement of material for formation of a whole. We feel that it is questionable whether or not the forced position was really maintained in this experiment.

Morgan

T. H. Morgan (1895) combined the Schultze rotation experiment with the Roux pricking experiment. He killed one half-blastomere of the frog egg by pricking and allowed the egg to develop either in a normal position--the dark pole uppermost, or inverted with the white pole uppermost. In the first case, he obtained only hemiembryos as had Roux. In the second

instance he obtained both hemiembryos and mostly complete embryos. Morgan believed that this result provided the answer to the conflict between Roux and O. Hertwig. Roux (1888a) obtained a half-embryo from a surviving half-blastomere, because he left the eggs in the normal position. O. Hertwig (1893) received a complete embryo because, according to Morgan's suspicions, he rotated the eggs accidentally. The rotation caused the surviving blastomere to lose its half-organization and acquire a complete organization through the shifting position of yolk matter and then thereby effected separation of the dead sister cell. The same should also occur without yolk shifting according to Morgan, if the pricked blastomere is completely destroyed. On the other hand, a shifting of the yolk does not always cause a complete organization of the half-blastomere, since a hemiembryo was obtained in one case with demonstrated yolk displacement. Yolk shifting cannot, therefore, be the sole factor influencing the formation of half or complete embryos. From these statements it follows, as Morgan considered it to be self-evident, that both twins in the Schultze experiment can be traced to the two first blastomeres.

G. Wetzel

O. Hertwig requested G. Wetzel (1895, 1896) to make a thorough check of the Schultze twin formation experiment by using the same methods on the egg of Rana fusca. He also obtained the same duplications as had Schultze, with the exception of one case of triplets. He was able to give /312 a closer analysis of the process of formation of Duplicitas cruciata, but he was not successful with the other forms.

One Duplicitas cruciata produced by Wetzel conformed very closely to that described by Schultze. This has been pointed out by Hey (1911, pages 186-187) and by Rudd and Spemann (1922). Schultze included such twins in the ventral-adhering group but, in reality, they represent typical cases of Duplicitas cruciata, exactly the same as Wetzel's case. The two forms differed only in that, in the case of Schultze's twins, the separate halves of the axial organs combined into one unit in the middle and at the posterior ends, comprising a medullary tube and a chorda, so that a typical Janus formation occurred (Figure 1a). From the unitary front end of each individual part, the axial organ halves deviate back-to-back in a fork from right to left or vice versa, in order to alternatingly join with the corresponding components of other individual parts. Such a formation was not produced in Wetzel's experiments (Figure 1b). Rather, each individual part has a unitary head, but the backs are split into Spina bifida (O. Hertwig, 1892) or they show, in Roux's terminology (1888a), an Asyntaxia medullaris. The dorsal ridges are fairly far apart in the rear and do not join reciprocally. The yolk mass, which otherwise separates the ridges of a Spina bifida, is here seemingly replaced by a complete embryo, in that each individual represents the yolk plug of the other. This may also be understood by stating

that: Two Spinae bifidae turn their backs on each other and are fused together by the protruding base of the gut (Figure 1b, Do). We also consider the very thorough description of ventral twins by Wetzel (1896, Plate I, Figure 7; Plate II, Figure 8 and pages 18-21) to be a typical cruciata formation, which corresponds to that described by Schultze. This consideration is based on the experience gained in our experiments.

The schematically drawn duplication in Figure 1 has been designated by us as the Schultze type of Duplicitas cruciata. Two primary unitary anterior ends or heads and two secondary unitary posterior ends or backs are characteristic of this type. The type of duplication described by Wetzel is only a subform of the Schultze type and differs from it in that no unitary dorsum is formed. This type is shown in Figure 1b, and we term it the Wetzel form of the Schultze cruciata type (or, abbreviated, the Wetzel cruciata form).

In Part V we consider Spemann type of Duplicitas cruciata, which is essentially different from both the Schultze type and its Wetzel form. The development process of such a Janus formation (i.e., a Wetzel cruciata form) is described in detail by Wetzel, whereby he bases his remarks partially on his own determinations, and partially on those made by other authors in another connection. The use of the latter type of determination is especially evident in his description of the formation of the characteristic structure of a double blastula, which he examined in sections and as a whole.

He agrees with Schultze in saying that one very often finds a white /314
streak on the vegetal or upper surface of the blastula in the region of the first cleavage (this streak is naturally visible even later). If this streak is not present, one may observe a whitish gray field of irregular outline. In every case where such a typical white streak is observable on the blastula, one may predict a duplication with certainty. On the other hand, duplications are also developed from blastulae, which do not present such a streak.

The outwardly visible white streak gives, according to Wetzel, an indication of the material distribution within the blastula. Just as the white streak on the vegetal surface separates two dark fields, one right and one left, the light yolk mass on the inside separates the pigmented plasma-rich material into two halves. If one cuts such a blastula across the white streak, Wetzel maintains that this conclusion may be proven (Figure 2). The relatively large yolk cells form a very irregular septum between the small-celled, dark material in the region of the first median plane of cleavage. This dark matter is located on both the right and left of the blastula. To the right and left of this septum in the vegetal half of the embryo (see Figure 2) there is found a small cleavage cavity on each side between the partition and the small-celled part.

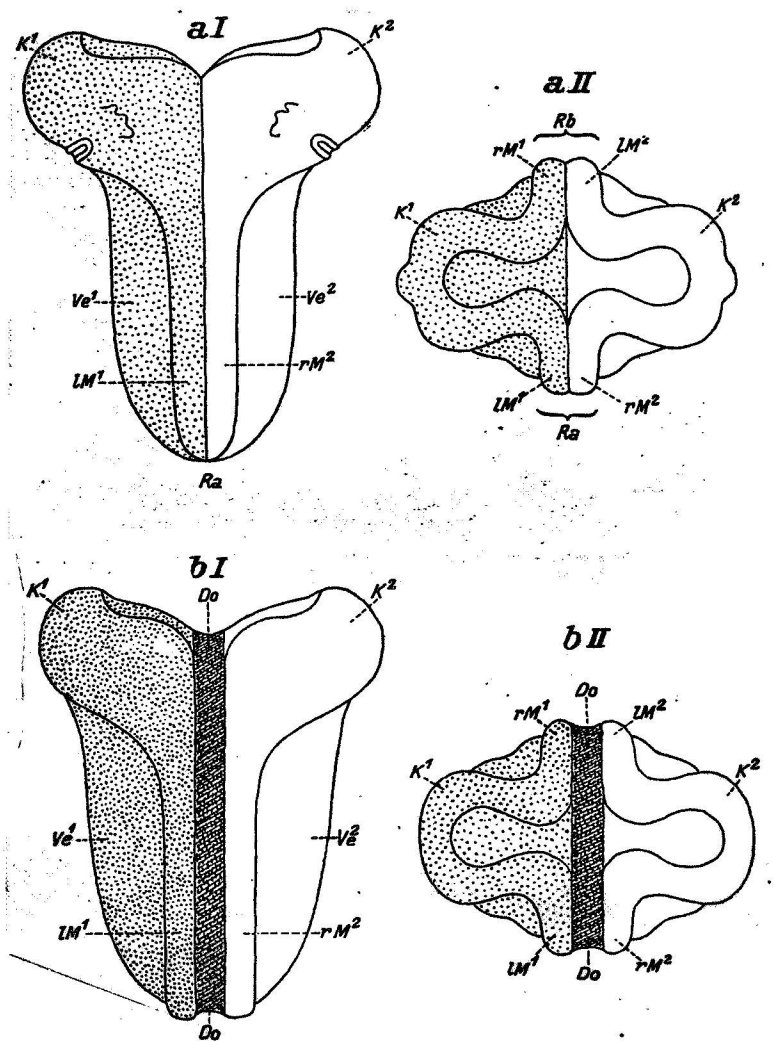

Figure 1. Schema of Duplicitas cruciata. a, Schultze type; b, Wetzel form of the Schultze type; in I, both from the side, in II, seen from the front end. One individual part is stippled, while the other is not. The component parts of one carry the exponent 1, the others carry the exponent 2. Each individual part consists of a head, or generally speaking, an anterior end (K^1 or K^2), a right and left medullary ridge (rM^1 and lM^1, or rM^2 and lM^2) for each, and a ventral side (Ve^1 or Ve^2). In Figure aI and bI only one medullary ridge may be seen for each individual part. Both are partially visible in Figures aII and bII. A suctorial disk and gill

(Caption continued at top of following page)

(Figure 1 continued from previous page)

anlage are indicated in the Schultze type. The Schultze type also shows two medullary ridges not belonging to the same individual part which have grown together into one medullary tube or dorsum, namely lM^1 and rM^2 to back Ra and rM^1 and lM^2 to back Rb. These backs or posterior ends are therefore secondary unitary formations, while all other parts of the duplication are primary unitary structures (head and ventral sides) (see Wessel, 1926). In the Wetzel form both individual parts are fused by yolk cells (Do) but nevertheless separated by them. No growing together of the individual correlated medullary ridges, which face each other, occurs. Each individual part constitutes a Spina bifida, which is implanted in the dorsal fissure of the other individual part by means of the yolk.

If a separation line (dotted line in Figure 1)[1] is drawn through this septum in the region of the first plane of cleavage the embryo is separated into two "symmetrical" halves, each of which is almost exactly the same as a normal blastula in structure. Their new axes are, however, perpendicular to the first plane of cleavage. Therefore, there are already two individual parts in the blastula. They touch each other with their new vegetal fields and are grown together within them.

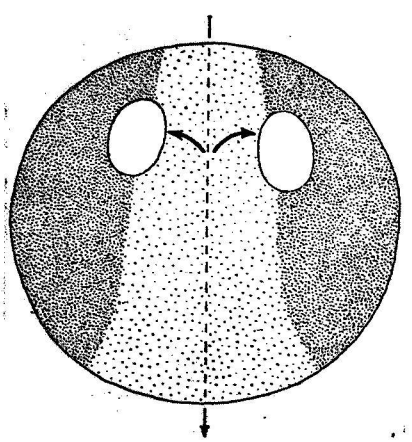

Figure 2. Explanation of Wetzel's opinion. This was drawn according to his Figure 20, Plate XXXIV (1895). The brown yolk cells are heavily stippled, while the white yolk cells are lightly stippled; further explanations may be found in the text

[1]Figure 2 probably intended.-Ed.

The occurrence of the described positions of white and dark matter was explained by Wetzel, with reliance on Born, without following the corresponding stages of development in detail by means of section series. The shifting of matter in the entire egg after rotation, which had been determined by Born (1885), is assumed here to occur in each half-blastomere. This shift does not lead to a complete 180° rearrangement, but rather, because the cleavage—the splitting of each egg half into cells—continues, this rearrangement is assumed to cease at the halfway point, so that then each half (i.e., each half-blastomere) in the blastula stage with respect to its substance would have the arrangement of an entire egg, whereby, however, the now vegetal portions of the original center of the egg are adjacent in the region of the first plane of cleavage and the animal portions lie on the original left and right sides. /315

The cause of the separate development of two individuals from one egg lies in the separation of the dark material into two halves by the white substances between them. This is then first the cause for the double blastula formation. This causes the formation of two major growth and cell proliferation centers, which now develop independently of one another, although they do cause mutual disruptions. The interposition of the yolk is the cause of the separation of the elements essential to the life of each half-blastomere from those of the other, namely the darkly pigmented matter. The separation of the two blastomeres is the cause of the duplication.

The gastrulation described by Wetzel is understandable from the opinion expressed by him that the blastula is a double individuum of the type mentioned. The gastrulation begins in the interior of the double blastula, approximately in the place and manner shown by the arrows in Figure 2. This blastula shows a double nature from the beginning. The gastrulation process may then be observed from the outside in the form of the meridianal cleft which is described in exact agreement with Schultze. This cleft was supposed to be formed by the invagination of inner material into the two cleavage cavities which would cause the vegetal material on top of them to sink inward. This sinkage would occur in the form of a long trough, whereby the gastrulation process which should be thought of as having a punctiform origin, could only run lengthwise in the direction of this trough as a result of the separation wall arrangement of the white yolk matter.

According to Wetzel, this is the only explanation for sections showing gastrulation stages with formation of this circular trough. He obtained such a section from an embryo he conserved in a stage where the gap covered 3/4 of the periphery and was then cut perpendicular to the middle part of the gap. This showed that two upper blastopore lips were present in the embryo. These lips are located opposite to one another within the embryo and each leads to a separate blastocele, which corresponds

to the location of the cleavage cavities on the blastula. These blastoceles have a common entrance from the outside through this gap. The gap is also, then, the result of the pushing together of the two upper blastopore lips and is therefore not itself a blastopore, nor it is a medullary tube. It represents the ideal separation plane of the two individual parts and serves as a sure external sign that a complete duplication will occur.

In order to prove the validity of his opinion that the key to the origin and formation of the Duplicitas cruciata lies in the special arrangement of the egg substances and their development Wetzel suggests the following experiments:

1. The pressing and rotation experiment should be conducted at the highest possible temperatures. Cleavage would presumably proceed so rapidly that no sufficiently large rearrangements could occur until the blastula stage.

2. Instead of high temperatures, low temperatures should be used. This would cause the cleavage to proceed so slowly, that a complete /316
rearrangement of the substances would occur before the cell division, which retards such rearrangement, has progressed sufficiently to effect such retardation. In both cases, according to Wetzel, not double embryos, but only single structures should result. A positive result, i.e., the formation of a duplication, would completely refute his hypothesis as to the inner cause for the development of twin formation. As far as we know, no such experiment or even one similar to it has been published as yet.

The occurrence of twins not belonging to the Duplicitas cruciata type is similarly conceived by Wetzel, who does not go into detail on this matter. He does assume that the same inner causal conditions, or similar ones, are the responsible factors. He is unable to offer any explanation for the formation of triplets, which occurred during his experiments. Based on his explanation of the splitting of the egg and the more or less complete isolation of the halves following this, the triplet formation must remain a puzzle.

Chiarugi

Chiarugi (1898) published only a short current account without illustrations concerning his experiments on Salamandrina perspicillata. He was interested in the abnormal phenomena observed during cleavage of the compressed and inverted eggs—namely the occurrence of pit-like recesses on the vegetal surface. We shall return to this in Part II. Chiarugi also indicates that the development of the egg may proceed in many different ways. In many cases, the course of development is normal.

In others, only slight deviations from the norm were observed at the beginning, without any distinguishable difference at advanced stages. Again in other cases the developmental anomalies were more distinct and "irreparable" but without special characteristics, or they led to the early death of the embryo, or to the formation of monstrosities that were complex and difficult to analyze. In about 20 percent of the cases Chiarugi obtained clear duplications. Anterior duplications were the most prevalent, those of the posterior variety occurring less frequently. He describes in addition one case which appeared to him as if a posterior end were implanted on the anterior end of an embryo. It is possible in this case that he observed a Duplicitas cruciata, which became a secondary unitary structure (see Part V, B4d). Chiarugi in his paper does not enter into particulars concerning the investigations of Wetzel that would have made recognition of such a Duplicitas cruciata easier for him: he also does not consider Wetzel's explanation for the origin of such duplications. He did feel that he had observed the gastrulation invagination occurring "at two separate points" and that the two blastopores later merged into a single linear blastopore gap. He concerns himself principally with the stages in which the medullary plate appears flat, and in which the double character is evident on it.

Tonkoff

Just as he had in the case of Wetzel, O. Hertwig suggested that Tonkoff (1900) repeat the Schultze experiment. Tonkoff conducted his investigations on the Triton taeniatus. The experiments were changed /317
not only through the choice of a different experimental object, but also insofar as the compression was conducted not immediately after insemination, but first in the 2-cell stage simultaneously with rotation.

He claims never to have observed a light streak on the blastula nor to have seen the circular cleavage during gastrulation. The gastrulation of the rotated Triton eggs, when observed externally, does not vary much from that of normal eggs. In two cases, typical duplications occurred in eggs, which had first been pressed and rotated in the 4-cell stage. This contradicts Schultze's statement concerning Rana fusca that a rotation conducted later than the 2-cell stage not only does not produce a duplication, but results in a wide variety of deformities of all types.

In his detailed study published in 1904, Tonkoff generally confines himself to a description of the inner anomalies of double embryos. He considered his most important find to be the fact that a completely harmonious, symmetrical twinning is seldom observed in Triton. It is more usual on the contrary that a medullary tube is very small and the chorda belonging to it is often entirely missing. He attempts to explain this with the assumption that it is very difficult to place both half-blastomeres under the same conditions in a rotation experiment.

A development center was formed in each half-blastomere, but no complete equilibrium exists between these two. Furthermore, the two half-blastomeres were not completely isolated from one another. In addition to this, these deviations from the ideal case of doubling would in no way contradict "the basic law governing the capability of the first blastomere for development into a complete embryo." (loc. cit., page 63).

Dürken

A number of other authors agreed with Wetzel's explanation of the occurrence of Duplicitas cruciata. These included O. Hertwig (1906), Hey (1911), Rudd and Spemann (1922) and Dürken (1919). Wetzel maintains that, at the beginning of gastrulation a meridianal furrow appears on the edge of the upward facing white field in the direction of the first plane of cleavage; this furrow gradually becomes a completely circular cleft, and therefore the "double invagination" began "at one point on the oridinal lower half of the egg" (1895, page 662), that is to say, in the vegetal portion now located at the top following rotation. Dürken (loc. cit., page 97) in a schematization that deviates from Wetzel's presentation, which of course corresponds to the actual findings, shifts the point of origin of gastrulation to two points relatively far apart from one another in the vicinity of the original animal pole (see his Figure 83). With reference to the entire group of figures, i.e., Figures 79-86, it is moreover to be noted that the designations a = animal pole and v = vegetal pole are used in reverse, since as a matter of fact as can be seen in Dürken's Figure 79, the upper pole marked a is the vegetal and vice versa.

In this presentation there are two points of view, which in our opinion are not substantiated, injected into the case by Dürken. In the first place, as Wetzel states, gastrulation began nearer the vegetal and not by the animal pole, as Dürken believes (loc. cit., Figure 82). Secondly, the term "double gastrulation" which Dürken borrows from Wetzel, should not be taken to mean that two widely-separated upper blastopore lips are formed. Dürken obviously uses these incorrectly constructed schemas to make the generally accepted assumption as clear as possible, that each of the half-blastomeres is capable of yielding a complete embryo. /318

Moszkowski

Moszkowski (1902) also discussed the method of occurrence of the Schultze-Wetzel duplication quite thoroughly, although purely speculatively. It is true that he did conduct the Schultze experiment himself and obtained thereby "a large number" (loc. cit., page 52) of Rana fusca

duplications, but he certainly did not study their genesis using his own material. If he had done so, he would certainly not have written such schemas for the development of the duplications, which contradict all the facts reported by Wetzel, which Moszkowski was by no means trying to undermine.

He cannot completely agree with the interpretation which Wetzel applied in general to the experiment. Wetzel's major cause for the occurrence of duplications was the separation of the dark substances in both blastomeres by the interposition of the dispersing white yolk matter. This is certainly true in the case of Duplicitas cruciata, but it is definitely not true for the other types of doubling. This opinion derives from the purest speculations. Even the development schema for Duplicitas cruciata given by Moszkowski does not correspond in the least with Wetzel's pictures and descriptions of the relative positions of light and dark matter within the rotated embryo.

In clinging to his belief in the dominance of the flow meridian, Moszkowski explains the occurrence of the duplication in the rotated 2-cell stage by stating that "the symmetry of the egg is disrupted, causing the interrelationship of the two cells to cease, with creation of a new symmetry in each of the two cells" (loc. cit., page 55). In the 4-cell stage, on the other hand, the cohesion of the masses and the internal friction is too great to produce new symmetry planes before the third cleavage begins. For this reason, only "totally asymmetric embryos" occur after rotation. After the third cleavage of inverted eggs, which have been rotated and held in this position, the eggs are naturally quite defenseless at first against the damaging influence of abnormally aggressive gravity force and can never become normal larvae (loc. cit., pages 56-57).

In a further experiment, Moszkowski (1903b) makes the following addition to this opinion concerning the development processes in frog's eggs. Based on the substance shifting in each half-blastomere, one point in each, or two in the entire egg, are "performed, at which the diminution of cells is inhibited earlier than in the remainder of the egg." (1903b, page 381). Primary elementary processes are begun simultaneously at two points in such an egg. Moszkowski understands this to be the gastrulation of frogs' eggs as a result of the growth of the cells and the differentiation within the embryonic anlage. From these two points the two heads of the duplication are developed. The determination that only Janus-like deformations resulted from the Schultze experiment agrees closely with this opinion. No Duplicitas posterior was ever observed. The occurrence of one would not have harmonized with his hypothesis that the differentiation emanates from two separate points. /319

One could admit the possibility of the fusion of two of a pair of individuals proceeding from two separate development centers, but it

would be unthinkable "that a simple, undivided development center could produce two individuals." (loc. cit., page 382). We should like to state at this point that, in our opinion, Moszkowski did not have a clear idea of just what a Janus formation was. He seems to include all duplications in this class if they have only two heads.

B. Statement of Problem

The original impetus in undertaking our experiments was provided by the following considerations. Roux (1885) stated that the fertilization meridian determines the plane of symmetry and that that side of the egg became dorsal that happened to be opposite to the side where the sperm was introduced. This would mean that the dorsal side would be determined only after fertilization. The validity of Roux's theory, which plays no role in the following reflections, is even more questionable than it was after the recent experiments of Weigmann (1927) and Banki (1927). In connection with Born's experiments (1885), Roux (1887) came to the conclusion that the flow meridian determines the direction of the plane of symmetry when the frog egg is in a very oblique constrained position. The idea is implicit, however, that that side of the vegetal field, which accidentally is nearest to the top in the oblique constrained position, will become the dorsal side. Furthermore, as a result of the sinking of the white yolk caused by gravity, only a thin surface layer remains and this forms the Born gray field.

Let us assume that Roux's opinion is correct (without considering the further advanced, but certainly refuted, opinion of Moszkowski (1902, 1903a and b) that gravity always determines the dorsal side in the manner described). By doing this, we are forced to reconcile them with the results produced by Spemann (1901-1903, 1914, 1918, and later). According to Spemann, a factor necessary for the formation of an embryo anlage is present only in the dorsal side. This factor was later recognized as the organization center and is already present in the plasma of the undivided and unfertilized egg. This leads to the explanation that, in the frontal position of the first cleavage in the constriction experiment, only the dorsal blastomere is capable of forming an embryo anlage, /320
whereas only a so-called abdominal piece without embryo anlage is produced by the ventral side. These results were not obtained with frog eggs, but rather with those of the Triton, but one cannot question the fact that they are valid for the frog eggs. This has subsequently all but been proved by the xenoplastic organizer transplantation between Rana and Triton carried out by Geinitz (1925). As may easily be ascertained, a contradiction exists between Roux and Spemann. According to Roux, any side of the unfertilized egg may become the dorsal side. Spemann maintains that the dorsal side is determined before fertilization by the localization of the organization center.

Furthermore, an inconsistency exists between Spemann's results and the manner in which the results of the Schultze inversion experiment are interpreted, as well as between this and Morgan's experiments. If the formation of duplications in the Schultze experiment is explained by the assumption generally accepted up to that time, which was that each half-blastomere received the organization of a whole egg, then each embryo anlage must be able to be traced back to a half-blastomere. If the first cleavage of a rotated egg is frontal, which is often the case due to the simultaneous pressing in the experiment, then one must also inquire as to whether or not the ventral blastomere of the rotated egg is capable of forming an embryo anlage. If this is true, one must assume that the ventral blastomere receives not only the yolk arrangement of the entire egg as a result of rotation, but also the organization center, which is normally located only in the dorsal blastomere. Schultze and Wetzel actually knew nothing of the position of the first cleavage with respect to the median plane at the time of their experiments.

Morgan (1895) killed one half-blastomere of the frog egg by pricking and obtained only hemiembryos by this process if the egg remained in its normal orientation. Whole embryos were most often produced, on the contrary, if the egg was rotated. He concluded from this, as we have already mentioned (page 5), that a half-blastomere yields a half structure when the yolk is oriented normally, and most often a whole structure, on the contrary, when the orientation is reversed. Morgan also gives no information concerning the position of the first cleavage in his experiments.

These experiments did not yield a decision as to whether or not the ventral blastomere of a rotated egg, in contrast to that of an egg in normal position, was capable of producing a whole structure. The possibility remained, however, that such might be the case. Spemann (1918) /321
indicated this and remarked that the positive result of an experiment conducted with this possibility in mind would destroy, as far as Rana is concerned, the validity of his results obtained with Triton. He did remark that the formation of an embryo anlage in the ventral blastomere could under certain circumstances also come from an inducing influence on the part of the embryo anlage in the dorsal blastomere, and that the ventral blastomere was not therefore necessarily capable of such a formation by itself.

In 1924, we obtained a typical duplication from one egg with a frontal first cleavage, which was then rotated. We felt that this proved the thesis that the ventral blastomere of a rotated egg was also capable of forming a complete embryo anlage. Several possibilities were open however for more accurate interpretation of these results. Either it was possible that the ventral blastomere of the Rana might also acquire an organization center, which would only happen under such special conditions as the Schultze rotation experiment, or it might also be assumed

that the shifting of the yolk substances could cause the reformation of such a center in the ventral blastomere.

This second possibility was feasible, if one agreed with Roux or Moszkowski that the determination of the dorsal side was definitely made by the flow meridian. Finally, it was not impossible that the explanation was contained in the above-mentioned conjecture made by Spemann.

Two determinations were necessary to resolve these questions. First, it had to be determined whether or not the two embryo anlagen in the Schultze rotation experiment always corresponded to the two half-blastomeres. Secondly, it had to be determined what relationship existed between the two individual parts of the twin formation and those regions of the egg, from which the normal formation of an embryo anlage originates. In this respect, the Rana fusca egg offers the great advantage that the region of the organization center is indicated by the presence of the so-called gray crescent, first determined by Roux (1888b). We had, therefore, to mark the location of the gray crescent before rotation and then to relate it to the orientation of the two embryo anlagen formed in the rotated egg.

To evaluate this properly, two further determinations had to be made. First, we had to determine what the relationship was between the two individual parts of the duplication. Schultze and Wetzel had described the manifold forms of duplication in Rana fusca, but only the formation of one of these types, namely the Duplicitas cruciata, was explained in any detail by Wetzel. A comparison of the great number of various types of twins observed by us in 1924 and 1925 showed us that, genetically speaking, the vast majority of them were Duplicitas cruciata. We published this information in a short paper (Schleip and Penners, 1925) and confirmed it more positively in 1926. Secondly, we had to determine just how the two embryo anlagen develop in relationship to the gray crescent. The corresponding experiments yielded manifold results. /322

First, the ontogenesis of the duplications showed surprisingly even more clearly than their comparison in the developed condition and they all belonged at least genetically to the Schultze type of Duplicitas cruciata (or to the Wetzel form of this type). This is reported on in detail for the first time in Part V of this study. Second, we proved definitely that, as a rule, the two embryo anlagen in the rotated egg originate with the same dependence on the gray crescent (organization center) as do the normal anlagen in the normally oriented egg. We reported on this briefly in 1925. Third, we found that the orientation of each of the two individual parts to the original region of the gray crescent, and therefore to the original polarity and bilaterality of the egg, varies tremendously, and that the formed organ anlagen have no relationship whatsoever to the original arrangement of the presumptive organ anlagen in the egg before rotation (Schleip and Penners, 1926).

Fourth, we were led to an examination of the gastrulation process in the rotated embryos. This resulted in only a partial confirmation of Wetzel's statements and we found some very interesting relationships, about which we made some suggestions in 1926.

Thus, the originally simple question concerning the generative potentiality of the ventral blastomere of a rotated frog egg led us to a thorough examination of the development of the various forms of the Schultze duplication. In our opinion, the results solve not only the original question, but are related to many other problems of a development-physiological nature, which were studied in the amphibian embryo by Spemann and colleagues and by Vogt and Goerttler using other methods. These problems will be discussed in their proper frame of reference.

The examination of duplications at the last stages of development using a prepared section was conducted by Miss Charlotte Wittmann and will be reported on separately. It will be necessary to mention them from time to time in this study, in order to illuminate some of our results. In Parts V and VI of this study, her results are taken into consideration fully, insofar as they are important to our general conclusions.

We must finally admit that we were not able to solve to our complete satisfaction all the problems that cropped up. The major reason for this lies in the fact that each individual duplication has its own particular characteristics which more or less differentiate it from all others. Its development could only be completely explained by means of section examinations. It must also be stated, however, that sections of earlier development stages cannot indicate with certainty all the details of further development.

C. Technical Information and Materials

1. Pressing and Rotation

As is well known, the Schultze experiment is simple and easy to conduct. The eggs are pressed between two glass plates in a position perpendicular to each standing axis. The pressure is applied to such a degree that the eggs, with their vegetal poles upward, cannot return to their original positions. The only difficulty in conducting the experiment is to find the degree of pressure that the eggs can bear without bursting, but at the same time will be sufficient to retain them in the rotated position. Schultze described the method he used exactly (see above). We used another method, which was more certain of yielding the desired results and which was more convenient to conduct.

A strip of plastilin is placed at each end of thoroughly cleaned and dried slides. Each strip is about as long as the width of the slide, approximately 1/3 cm wide and 1-1/2 times thicker than an egg. A small paper strip bearing the experiment number is placed between the glass and the plastilin block on one end of the slide in such a manner that the inscribed side of the paper faces the glass. A large number of slides are prepared in this manner. The eggs are then artificially inseminated according to Roux's method. The first cleavage begins on the average about three hours later.

About 15-30 minutes before the eggs have reached the stage where they should be rotated, one egg is placed in the middle of the slide in its gelatinous covering. If the gelatine is sufficiently swollen, little or no water need be added. In a short time, the eggs will have rotated with their vegetal pole downward. At this time, another well cleaned and dried slide is placed on top of the plastilin block and pressed lightly, causing the egg to be compressed gently, so that it may still orient itself in its normal position.

This first light compression is conducted because the eggs tend to /324 turn slightly when pressed more heavily at first, so that their axes are more or less oblique to the pressing surface. These preparations remain in this position until the first division has reached the desired stage. The egg is then subjected to the final pressing under the control of a binocular magnifying glass by pressing the ends of the upper slide firmly against the plastilin block and thereby against the lower slide with the thumbs. One soon learns the approximate degree of pressure to apply which may still be withstood by the eggs, which can vary rather significantly, of course, with eggs from different females and in different stages of division. One must use the heaviest possible pressure, because otherwise many eggs rerotate to their normal positions after the slide is rotated. This causes the loss of about 1/4 of all the eggs used in the preparation of such slides, since overly strong pressure causes them to burst. All slides which have been properly prepared are then turned over, so that the vegetal side of the egg is upward and the experiment number on the paper may be read.

We usually placed 2 or 3 eggs on one slide and differentiated between them by using the letters l, r, or m in the control designation. This enabled us to increase substantially the number of usable eggs without preparing more slides and saved much time in handling. We should mention at this point that all eggs later were given new designations, in place of their original number, for ease in checking.

As a result of the pressing of the original upper slide against the plastilin block, not only were the eggs pressed, but also the two slides were bonded together by the plastilin. This enabled us to dispense with the rubber band used in Schultze's method of preparation.

The procedure is different if one desires to press and rotate the eggs at such an early time that the eggs cannot orient themselves within their perivitellin space to their normal position. In such cases, the eggs must be removed from the uterus and placed immediately on the slide with its plastilin block without using water as exactly as possible with the vegetal pole upwards. Next, water containing a drop of sperm is added to the egg, and at the same time the plastilin block is applied to the paper strip with the experiment number upward. Then the second slide is laid on the plastilin block, lightly pressed, and finally the whole preparation is turned so that the eggs are in a normal position. At the desired time, pressing and finally rotation may be carried out. Only a small percentage of eggs, which are pressed at a time too soon after insemination, develop.

2. Further Cultivation of the Embryo

The eggs remain in the rotated, compressed position at room temperature or, if one wishes to retard the development, in ice water or in a refrigerator. To prevent the drying of the gelatin, the preparations /325 may be placed in flat saucers with water, which may have ice cubes added for cooling. If one wishes to conduct the marking described below, it is necessary to use another method, which is quite as easy as the first. One fills the space between the two slides with water from a pipette. The water will not evaporate much overnight in a heated room, even when one takes no special precautions. Naturally, however, the slides can be placed in a damp chamber. On the next day (it is not necessary to be too careful about keeping within certain time limits) the eggs are released from the press and placed with their control numbers in individual water-filled saucers.

This method of achieving the Schultze duplication is so easy and convenient that we recommend its use in classes, so that the students might have the opportunity to directly view the impressive phenomenon of the formation of duplication from a single egg.

3. Marking the Direction of Bilaterality of the Egg and the First Cleavage

We marked the direction of bilaterality and the first cleavage of the egg of Rana fusca in the following manner. On material, which showed the gray crescent with sufficient clarity, the dorsal direction was marked on the slide, which lay on top after rotation, with an inked arrow as an extension of the median plane of the egg in the dorsal direction.

The first cleavage, if it lay toward the middle, was indicated by a line across from the arrow on the ventral side of the egg, or when it

lay elsewhere, through two lines in the extension of the direction of cleavage. This last case is illustrated in Figure 3. When some time has elapsed after rotation, it is then possible to check to see if the egg has rotated in spite of pressing. The determination of the position of the gray crescent and therewith the bilaterality may be conducted very satisfactorily in many cases, but is fraught with difficulty in certain other instances. We attempted to avoid instances of the latter type. Whoever has tried to determine the position of the gray crescent on Rana fusca eggs will have discovered, in spite of practice, that now and then he is mistaken. The worst aspect of this is that one cannot compensate for such unnoticed mistakes afterwards, because the gray crescent cannot be recognized soon after rotation. The determination of the gray crescent is easier in those eggs which are often seen to have a more or less conspicuous dark spot in the middle of the white field on the vegetal pole (Born 1885, Weigmann 1927).

4. Examination of the Living Embryo /326

The development of the living embryos was followed by means of a Greenough stereoscopic microscope, using good overhead light. Embryos with marked direction remained undisturbed in the press until the blastopore groove or another blastopore structure was more or less developed. The marks on the slide facilitated the determination of the orientation of the blastopore with reference to the direction of bilaterality and the first cleavage. Continuation of pressure did not inhibit gastrulation or the anlage of the embryo. This was true of rotated embryos and those in normal position. It did retard them, however, possibly modify them, and tended to make them less distinct. For this reason the pressure was released as soon as the appearance of the blastopore anlage offered the possibility of determining the direction of the embryo with reference to the main direction of the egg and the first cleavage. After the slides are loosened, the flat-pressed egg immediately becomes more spherical and therefore seems smaller (for this reason the earlier stages shown in our illustrations are mostly larger than the later stages). The embryo maintains its position during this process since the gelatinous covering still adheres to the slide and the embryo has not as yet turned inside the gelatinous covering during this period. The marks on the slides after their loosening therefore are still valid for the direction of bilaterality and the first cleavage.

We checked constantly by comparing the embryo's orientation to the markings before and after the loosening of the slides. This enabled us to remove from the experiment all those eggs that had changed position as a result of unskillful release. The embryo remained in its position between the two loosened slides, and developed very well under conditions of little or no pressure. Drying out was prevented by keeping the space

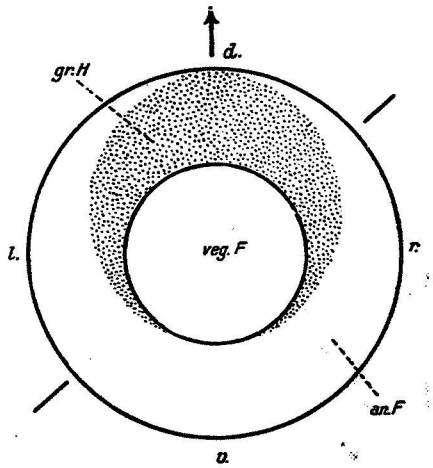

Figure 3. Schematic of the localization of the regions of the egg or the blastula, as well as the direction of the first cleavage by means of suitable markings. The arrow points in the dorsal direction. veg F = vegetal field; an F = animal field; gr H = gray crescent; d = dorsal; v = ventral; r = right; l = left. The first cleavage here runs oblique to the median. If the first cleavage was median, only one line would have been drawn opposite the arrow

between the two slides water-filled at all times. This method of cultivation between two slides has an advantage over cultivation in saucers in that it enables one to observe both the animal and vegetal surfaces of the embryo without the danger of damage by tearing the gelatinous covering. This possibility is always present when cultivation of a free embryo is conducted in a saucer.

Sooner or later, after the embryo anlage appeared, the embryos were removed from the chamber and placed in saucers. This was naturally done earlier with those embryos in which structures appeared on their edges, which could not be observed using the storage method described. The storage of the embryos in saucers is by no means more protective than the method we used with the slides, since it is often necessary to turn the embryo back and forth with the gelatin under the microscope, in order to obtain a clear picture. This, of course, means that tearing and stretching of the gelatin cannot be avoided. This tearing and stretching, in turn, exerts tensions on the vitelline membrane, which can often have a seriously damaging effect on embryonic parts. It often occurs that the vitelline membrane is pressed against some projecting portion, possibly an end of the body, causing it to turn white and die within a few seconds. /327

It is not always easy to relate the directions of older embryos to those of their early stages. These embryos often show the most remarkable changes in form (very noticeable folds and furrows) which can dominate the entire picture, only to disappear without leaving a trace a few hours later. The trough of the blastopore is no more dependable a mark. It can extend itself or close itself off into a number of sections, so that it is no longer visible. It can also change its position entirely, just as a rubber band can around a ball, constricting and sliding to one side. We were unable to conduct vital staining.

As determined by Vogt (1925), Nile blue sulfate was not suitable for use with Rana fusca, and the otherwise acceptable neutral red did not color the darkly pigmented portions of the upper surface adequately. A series of orientation experiments that we ourselves arranged confirmed Vogt's opinion. The orientation concerning the embryo may, however, be achieved in the following manner. First of all, the distribution of light and dark areas on the surface, as well as other accidental markings, may often be helpful for information concerning the direction of the embryo; occasionally small artificial marks may be made by pricks. Secondly, every embryo, the development of which is to be observed to the end, must be checked and drawn about every four hours.

The development to definite evidence of a duplication lasts about 60 to 70 hours after the start of gastrulation. The embryos must be kept under constant surveillance during this period. Meanwhile, contrary to Schultze's statement, it is not possible to grant oneself a rest period of about six hours at night. This is especially not practicable because each individual check occupies a considerable period of time and a number of individual embryos, usually 15 to 30, must be examined in each experimental series. This is necessary because certainly not all the embryos develop favorably into precisely the desired forms. As an added factor, we could not relieve each other in the successive experiments because the investigator must know his material exactly from the beginning to the end of the experiment. The researcher need not have personally prepared the embryos to the point of gastrulation, but he must be present during the remainder of the experiment. Furthermore, we wish to emphasize the fact that the spawning time of the gray frog is very short. We were able to alleviate this circumstance somewhat by first using frogs from the Wurzbürg area and then switching to frogs from the Red Moor area of the Rhön, where the brown frog begins to spawn somewhat later than the lowland frogs. Taking all this into consideration, it is not astonishing that our experiments concerning the various types of duplications had their limits.

5. Summary of the Embryos Examined

We were able to conduct a number of individual experiments in the spring of 1926, which were partially based on our experiences in the

spawning time of 1924 and 1925. Table 1 gives information about the scope and results of these experiments. In 1926, we examined a total of 287 embryos from 9 different females. Of these, 158 could not be included in the final evaluation since they either died prematurely (50) or could not be analyzed fully (108). The incomplete analyses were primarily due to loss of orientation during some stage of development. We were able to fully analyze 129 individual embryos. /328

To these successful individual experiments of 1926, we added a few others from the spawning season of 1925, during which we conducted primarily mass experiments with however a few individual observations. The experiments conducted in the first spawning season (1924) on Rana fusca and esculenta, as well as on Triton taeniatus, alpestris and cristatus, may only be given orientation value according to our present state of experience. They were, however, of great value to us since they began to show us the inadequacies of all the earlier experiments made by other authors. /329

We conducted the rotation experiment successfully with Rana fusca and esculenta, and with Triton taeniatus, alpestris and cristatus. The eggs of the first two types of Triton were most suited to the experiment in that the duplications developing from them are best capable of life, and a relatively large number survive and attain an advanced and flourishing stage of development. The Rana esculenta eggs are the more delicate of the two types of frog eggs. The duplications developing from them live a few days at most. In spite of this disadvantage, we finally decided to limit our experiments on the Schultze duplication to Rana fusca. The sole reason for this decision was the fact that the gray crescent in this form of egg allows for sufficiently exact determinations of the direction of bilaterality. The dorsal side of the Rana esculenta eggs may also be determined easily, but, as has been stated, these eggs are too delicate. Vogt (1923 b) claims that the dorsal side of the blastula of Triton eggs may also be recognized by the pigmentation, but this determination could not be made reliably in this connection.

6. Section Examinations

To examine the inner processes of development in the cleavage stage, as well as in the blastula and gastrula, we prepared a large number of section series. In general, we followed the suggestions of Weigmann (1927, pp. 52-54) in the fixation, imbedding and cutting of these sections. It will therefore be necessary to add only a few remarks here.

We found that it was not absolutely necessary to maintain the times suggested by Weigmann for imbedding (sic) our slides in 96 percent alcohol and more in the individual mediums. We usually exceeded his times by more than double. It is important that only absolutely

Table 1

Female	I	II	III	IV	V	VI	IX	X	XI	Totals	
1. Dead without result	9	3	1	10	11	9	2	-	5	50	158
2. Unclear (including duplications	6(6)	11(8)	5(3)	14(13)	12(7)	25(20)	17(16)	12(3)	6(4)	108(80)	
3. Duplicitas cruciata typica	2	9	-	8	1	5	2	9	2	38	129
4. Duplicitas posterior	-	1	1	1	1	2	2	2	1	11	
5. Duplicitas anterior	1	4	-	4	-	-	-	-	-	9	
6. Duplicitas ventralis	-	2	-	1	1	5	-	-	-	9	
7. One posterior and one rudimentary head	1	1	1	-	4	1	3	1	-	12	
8. Spina bifida	3	1	-	12	-	-	-	-	-	16	
9. Primary unitary formation	4	1	1	-	1	3	3	1	-	14	
10. Secondary unitary formation	2	1	1	1	2	-	1	-	-	8	
11. Special cruciata forms	-	-	-	-	1	5	-	-	1	7	
12. Homogeneous formation, genetically not a cruciata	2	1	-	-	1	-	-	1	-	5	
Grand totals	30	35	10	51	35	55	30	26	15	287	287

Note: All embryos under numbers 3-10 were considered by us to be cruciata forms, at least genetically, of the Schultze type. All embryos under numbers 3-7 and 11 are certainly duplications (from numbers 8, 9, and 10, an additional series). This is a total of at least 86.

clean and filtered paraffin be used in imbedding and the blocks should be allowed to lie for weeks before cutting. The thickness of the sections was 15 μ. Coloring was accomplished in Delefield and Orange G. Cleavage stages, blastulae and gastrulae were cut latitudinal and parallel to one meridian, usually perpendicular to the white streak, if it were present, and to the groove.

7. Remarks Concerning the Figures

The drawings of the living embryos were made freehand with the use of a magnifying glass. This could lead to some errors in the representations of the proportions of the embryo, but not errors which would be significant in the evaluation of the process of development. It was not possible to sketch all the details, especially the exact indication of the distribution of light and dark spots. We therefore limited ourselves to marking the area of the light or white streaks or areas with /330
a series of dashes and the boundaries of completely or partially white zones with dotted lines. On most of the text figures, the direction of bilaterality and the first cleavage are marked in exactly the same manner as they are on the slides.

In Figure 5a, for example, the line and the arrow give the location of the first cleavage. The arrow points in the dorsal direction; therefore, the first cleavage was medial in this embryo. In Figures 3 and 8a, the two lines show the location of the first cleavage. Since the arrows once again point in the dorsal direction, the first cleavage was oblique or frontal. The directions are given in the same manner in the photographs of Plates VI and VII. Where no markings are present, the dorsal side faces upward and the direction of the first cleavage is median (see the explanation of Plate VII, Figure 14).

On many pictures (sections) the right and left sides of the egg are designated with r and l, the dorsal and ventral sides with d and v, and the animal and vegetal sides with an and veg. These designations are always based on the direction of original bilaterality, where they were recognizable by the arrangement of pigmentation of the egg before yolk shifting. As Figure 3 shows, one can recognize approximately the location of the main regions of the egg or the blastula from the markings. The original white vegetal field occupies the middle part of the vegetal side and is surrounded by the region of the original gray crescent (stippled), which is widest on the dorsal side and whose "horns" decrease in size on both sides in the ventral direction.

The remainder or the visible portion of the embryo and the entire animal surface is the original animal field. The successive stages of development of one embryo are marked with a, b, c, etc. These letters have no exponents when they are shown in the vegetal side of the embryo.

They carry the exponent 1 (for example a^1) when they are shown on the animal side of the embryo. On the edges or in other views of the embryo, which are always represented more exactly, they carry the exponent 2.

If 2 has already been used, the exponent 3 is added (for example a^2, a^3, etc.).

The rearrangement of the yolk matter during cleavage after the rotation of the egg, as well as the formation and development of different blastopores, has been photographed in a series of pictures (Plates VI and VII). A number of difficulties prevented us from following in the same manner the entire development up to the formation of the embryo anlage. The photographs shown are completely unretouched and therefore some light air bubbles appear as light, circular spots on some of them. The photographs of the sections in Plates VIII-XI were retouched only slightly in certain cases. When we were forced to retouch slightly (Plate VIII, Figures 26a and 27; Plate IX, Figure 30; and Plate X, Figure 47), it was only when the monochromatic photograph showed too little of the double-colored section. We suggest that the pictures be viewed with the aid of a magnifying glass. /331

PART II. CLEAVAGE AND REARRANGEMENT OF THE YOLK

The Schultze experiment is a twofold one. First, the eggs are pressed between two slides which are perpendicular to the egg's axes. Secondly, the portion of the egg containing the heavy, white yolk (the vegetal half) is rotated to the top and held in that position.

The pressing process, when performed in the prescribed manner, causes a change in the cleavage divisions. The planes of the first two cleavage divisions are not disturbed. They run meridianal and perpendicular to one another. The percentage of eggs with frontal first furrows is increased if the eggs have been pressed or just lightly pressed (see above) a long time before the beginning of cleavage. The third furrow is not, however, a latitudinal one, as would be normal, but also a meridianal or perimeridianal, since it is also perpendicular to the pressing surfaces (Plate VI, Figure 1c). The further effects of pressing on the course of cleavage need not be treated here, since sufficient material has already been presented by others; notably Born (1893). We should like to remark only that, beginning with the fourth division, the direction of cleavage is increasingly varied.

The 180° rotation of the egg and its forced position in this orientation has the effect that the force of gravity has the capacity to influence the yolk substances, which vary in heaviness. Since the egg cannot rerotate itself, the heavy white yolk matter on top sinks, thereby forcing upward in the same manner the lighter, brown yolk substances.

This rearrangement of the substances caused by the effects of gravity was described in detail by Born (1885), but only insofar as it occurs within the egg before the first division. In the Schultze experiment the rotation is conducted during or after the first cleavage, whereby other conditions are produced for the yolk rearrangement. Basically, nothing more can occur in the halved or quartered egg that cannot take place in the whole egg. In general, each half or quarterblastomere corresponds in structure to a whole egg. The two or four blastomeres contain vegetal white and animal brown yolks, and the first of these must sink, while the second rises, in the forced position. Since, as we have already stated, the furrows of the third stage of cleavage run meridianally because of the special type of pressing, nothing stands in the way of the rearrangement, even in the 8-cell stage. As soon as furrows appear latitudinally, however, a sinking of materials from the

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upwardly directed vegetal half into the animal half that lies below and a corresponding ascension of lighter materials cannot occur. By the time this stage occurs, the white yolk matter has already sunk quite far, although it has not by any means completely collected at the bottom. From this point on, there can only be yolk rearrangements within the 1/16-blastomere, and this is stopped very quickly by further division.

When attempting to trace the course of yolk rearrangement during the Schultze experiment more exactly, one is faced with the difficulty that each embryo has its own special characteristics in this respect. For this reason,it would be impossible to collect a series of embryos of different ages, in which the yolk rearrangement had run its course in exactly the same manner, but which was at different stages at the time of collection; a study of this series in section would yield an exact picture of yolk rearrangement. This is even more unfortunate, because the form assumed by the duplication as it is later developed depends indubitably on which rearrangement of the yolk has occurred during the experiment. Generally speaking, there is no difficulty in obtaining, in a general way, a picture of this rearrangement.

1. Superficial Aspects of Eggs in Cleavage (Plate VI, Figures 1-3)

We were able to photograph the changes in the arrangement of yolk substances in a large number of eggs, as manifested by the superficial /333
aspect of the eggs. The following are some examples.

Figure 1a shows an egg which was pressed and rotated during the first cleavage division. The first furrow, in a median position, begins to encroach upon the vegetal side from the dorsal side. The vegetal, pure white field may easily be distinguished from the darker gray crescent which is widest dorsally (at the top of the picture), and which is symmetrically divided there by the first furrow. The second cleavage division was completed two hours later (Figure 1b).

The second frontal furrow had divided each half-blastomere into a smaller dorsal (top part of the picture) and a larger ventral cell. The extension of the pure white field was substantially decreased. The area of the middle region of the gray crescent could still be recognized fairly easily by the gray coloration. A whitish mass, covered partially on the surface with pigment, lies at the vegetal pole. The first furrow, which intersects the vegetal pole, was widened at one point into a hole-like formation, which appears as a dark spot in the picture. Two and one-half hours later, the third division had been completed (Figure 1c).

The furrows ran perpendicular to the pressing surface, in agreement with Born. The region of the gray crescent could no longer be distinguished. Pure white areas were found only in limited dimensions

in the four blastomeres surrounding the vegetal pole. The point of intersection of the first and second furrows was deepened into a hole-like formation, and dark pigment was located in a direction ventral to this formation. This dark pigment was obviously derived from that seen on the white complex in Figure 1b at the vegetal pole. These pictures give the impression that the white yolk on top had sunk both in the direction of, and around, the line of intersection of the first two furrows, whereby the hole forms on the vegetal pole between the blastomeres. Furthermore, one gains the impression that the rising brown yolk matter collected concentrically above the sunken white yolk matter. The slow cleavage of this embryo was caused by severe cooling. Because of the interchange between this severe cooling and the elevation of temperature caused by the intense illumination needed for photography, this embryo died when it was still large-celled.

Figure 2a shows a pressed and rotated egg at a stage where the first furrow, which also ran in a median direction, was almost completed. The vegetal pole is marked here by a dark pigmentation within the white field (see Born 1885, and Weigmann 1927). The middle region of the gray crescent, which faced upward, may be differentiated easily from the remainder of the surface. The second cleavage was almost completed after 32 minutes. /334

The second furrow is distinct at this stage, whereas the first furrow is a very small split, which, however, may still be recognized in Figure 2b. The region of the gray crescent was no longer clear and the white field was greatly decreased in size. In its place there is a bright white complex extending in the direction of the first furrow. A few minutes previous to this, the vegetal pole was concave, and it must be concluded from this as well as from Figure 2b that the white yolk matter had sunk along the line of the first plane of cleavage and not around the first axis of cleavage as in the previous embryos. Thirteen minutes later (Figure 2c), we were able to observe the white material on both sides of the median white strip, flowing toward it. This strip became more and more clear and was easily differentiated from the less white region surrounding it. The second, very deep furrow had just been completed 25 minutes later (Figure 2d), and the third meridian furrow was almost completed.

The pure white zone directed toward the median was no longer, but somewhat wider, while the whitish area surrounding it was not quite as large and had already been clouded by the more darkly pigmented brown yolk matter, which collected above it. After completion of the third division (Figure 2e) 20 minutes later, the pure white zone had become even wider and had turned into an irregularly outlined white spot on the vegetal pole, which could no longer be sharply differentiated from its surroundings. The 8 meridianal furrows, obviously in connection with a new cleavage, became more clear 50 minutes later (Figure 2f).

These furrows cut especially deep into the pure white zone, causing the formation of the irregular hole-like recesses. Cleavage was in an advanced stage of development one hour later (Figure 2g).

Thin strips of dark pigment could be observed at a number of points along the cellular borders in the white zone. Some small pigment islands could also be seen at other points. We were unable to determine the origin of this pigment. Since some of the cellular borders appeared at this stage to be very white lines, overlaid by these pigment lines, it seems that the white material still sinks principally along the lines of the cell septums of the many small blastomeres. The brown pigment, which flows from the sides toward these cell dividing walls, forms small pigment lines or granules, which do not sink with the white yolk matter. After another hour, no other changes could be observed with the exception of the further progress of cleavage.

One recognizes, however, that the vegetal cells (Figure 2h) are clearly smaller than the animal (Figure 2h^1), which corresponds to the information furnished by earlier authors (see Historical section). The animal side remained completely, darkly pigmented and did not change later. /335

The vegetal side, apart from its edges, was light brown and showed a few lighter to all white spots in the small-cell blastula stage (Figure 2i) 11 hours later. Some of these light spots could clearly be related to the corresponding areas of earlier stages (see Figures 2i and 2h, left side). At this stage, size differences between animal and vegetal cells could be determined no longer. According to the discussed surface pictures of this embryo, one must come to the conclusion that the white yolk matter did not sink around the cleavage (or egg) axis uniformly, but rather preponderantly along the plane of cleavage of the first two blastomeres. It must be anticipated, therefore, that this white matter would not form a sort of round column within the egg, but rather an elongated mass moving in the sagittal direction, at least at first, meaning in the vegetal portion of the egg.

Since only a rather poorly outlined, light-colored region of white yolk matter remains in the surface aspect of the egg, it would seem that it would form an irregular round mass within the egg, or at least that it would not form a partition running in the direction of the first furrow. We did cut this embryo in the blastula stage, but the cut was unfortunately meridianal, so that this series could not yield any absolutely certain results with respect to the form of the white yolk matter inside the egg.

Figure 3a shows an egg, which has just been pressed and rotated, with a frontal first furrow. The gray crescent, which cannot be clearly differentiated from the white vegetal field in this instance, is pointed upwards in the photograph. In Figure 3b (3-1/4 hours later) and Figure

3c (after a further 100 minutes) one may observe the yolk rearrangement in the surface during cleavage. As in the first two examples, the size of the vegetal white field decreases and hole-like recesses appear on the vegetal pole in the areas of cleavage and the cleavage intersections. At first it was not externally to be determined that the white matter sank along the border between the first two blastomeres. One does see in Figure 3c that the white field extends somewhat in the direction of the second furrow. The embryo presented a similar appearance 9 hours later (Figure 3d). Finally, (Figure 3e, after another 7 hours) a blastula had been formed which had a pure white streak and a lesser white band through its vegetal surface; a white band exactly as had been /336
described by Schultze and Wetzel. This was the only embryo photographed during cleavage which shows a good formation of such a band. Further discussion of this phenomenon will be conducted in the next section of this study.

The discussed examples, as well as the other embryos we photographed and followed, do not show anything not reported on by earlier authors, but they do show pictures of the changes of the embryo's surface for the first time. We concluded from these pictures, just as did earlier authors from their examinations, that the heavy, white yolk partially sinks and the space formerly occupied by it is taken up by brown yolk matter. It cannot be concluded from these pictures or from those not shown that the white yolk always sinks along a single plane of cleavage such as the first or second occurs. It is possible that, as in the case of the last embryo discussed, the white yolk sinks in the form of a round column around the axis of cleavage. As will be shown later, the white yolk matter often slides along the outer surface.

It would seem that many of the noticeable phenomena appearing on the surface are not significant in the further course of development. The hole-like recesses appearing in the region of the vegetal pole, which we found to be especially pronounced in Urodela eggs (Triton) (as stated by Wetzel and Chiarugi), seem to be based solely on the changed surface pressure of the blastomeres. The pigment lines, which are sometimes seen running across the furrows and which later disappear, can hardly be related to the blastopore groove which is first recognized as a pigment line.

It seems to us to be important that the sinking of the white yolk matter seems to continue even after the egg has been divided into 4, 8, or more blastomeres. In this case the yolk rearrangement can only be accomplished within the individual blastomeres. As will be discussed in Part IV, the arrangement of the white material visible on the surface certainly has great significance for the further course of development.

2. The Rearrangement of Yolk Substances According to Section Pictures (Figure 4) /337

The cortical layer of a normal Rana fusca egg contains a thin, but very dense, layer of pigment spread over the entire animal field. It is also intensively pigmented in the region of the gray crescent, but this layer is even thinner and somewhat more disaggregated (Roux 1903, Weigmann 1927). The vegetal field is unpigmented. Roux (1887) furnished the most exact information concerning the distribution of the yolk substances. We should like to refer to his picture (loc. cit., Plate V, Figure 5) and confine ourselves only to the following remarks.

The white portion of the yolk (i.e., that portion not containing pigment) lies in the vegetal part of the egg and consists of rather large lumps or layers mixed with a small amount of plasma. The animal portion of the egg is filled with brown yolk matter (i.e., yolk containing pigment granules) which also partially encases the white yolk matter at the sides. It consists of smaller lumps between which less plasma may be found than between the lumps in the white yolk.

When one fixes and divides sections into series, which show pressed and rotated eggs in various stages of cleavage, one may thereby gain an idea concerning the course of yolk rearrangement. As we have already mentioned, this idea will only represent an approximation, since each egg shows its own particular characteristics in this respect. Figure 4a shows a meridianal section of an egg in the 4-cell stage. The section plane is perpendicular to the second furrow, while the first furrow, cut on a tangent in the animal half of the egg, is recognizable in the lower part of the picture. The white yolk matter (light-colored) is clearly seen as a compact mass, which has sunk perpendicularly to the bottom. It is still in contact with the whole vegetal field, however, and has not reached the animal side of the egg. There the brown (dark) yolk matter has only risen partially along the periphery of the white yolk.

Figure 4b shows the same type of section, which was cut through an 8-cell embryo. The white yolk forms an axial strand extending from the vegetal to the animal side. The brown yolk has risen toward the animal side in each of the eight cells, principally in the axis of each of these cells. Latitudinal cleavage must stop the further ascent of the dark yolk, as shown in Figure 4c. The distribution of brown and white yolk in this egg, that presents lateral cleavage, is almost the same as in Figure 4b. Elongations are possible from now on only within the animal and vegetal cells. Figures 4d and 4e show the arrangement of the two types of yolk in more advanced stages of cleavage. The following remarks should be made concerning all of these pictures: /338

The white yolk has sunk to the egg cortex of the animal surface in those embryos, from which Figures 4b, 4c, and 4e were taken. In Figures /339

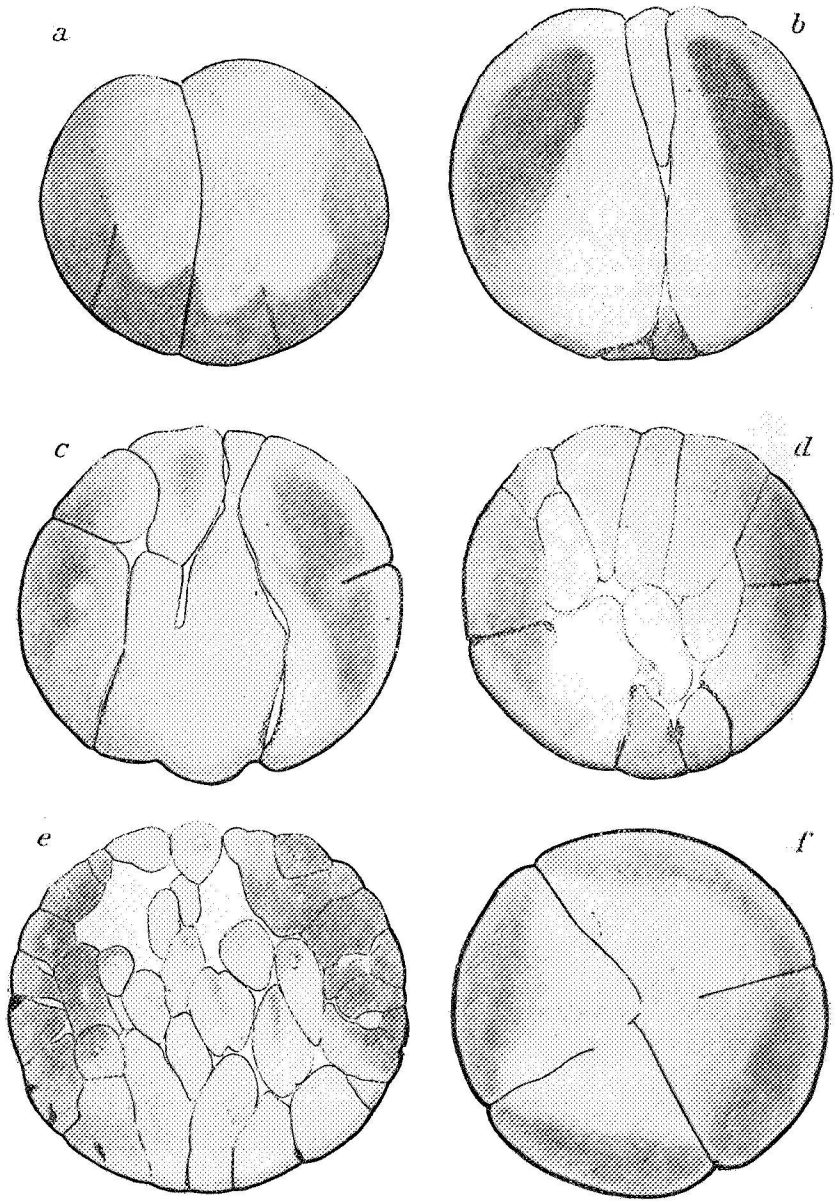

Figure 4. Semischematic of sections of cleavage stages of rotated eggs. a-e, meridianal sections; f, latitudinal section; white yolk, light-colored; brown yolk, dark; pigment, black

4a and 4d, a relatively large amount of brown yolk still remains in the animal portion. This difference is explained by the experimental conditions used. The first group of eggs were placed in ice water immediately after pressing and rotation, which severely retarded their cleavage.

For this reason, the white yolk was able to sink to the animal side and collect there before the latitudinal furrows had formed. The other eggs developed at room temperature, so that the cleavage proceeded more rapidly than the reorientation of the yolk, with the result that the major portion of brown yolk remained at the bottom. It should be mentioned that duplications occurred regardless of which experimental conditions were used. As far as we were able to ascertain from our observations, which were not statistically evaluated, the occurrence of duplications was about equal for both types of experiments.

The experiment suggested by Wetzel, which is mentioned on page 10, was therefore conducted with at least partially positive results. The occurrence of duplications is apparently not affected by the degree to which the white yolk has sunk. In none of our experiments, however, had the white yolk sunk completely to the bottom, as Wetzel thought it should. We feel that it is questionable whether or not this may be effected through the force of gravity, since it is not possible, without damaging the egg by too severe cooling, to retard cleavage until the white yolk has completely collected at the bottom. This experiment might, however, be conducted successfully if centrifugal force were applied. It should also be mentioned that we allowed Rana fusca eggs to cleave at elevated temperatures in the incubator. Duplications were also formed under these conditions, but we were unable to ascertain whether or not the yolks in such eggs, as one might assume, merely descended slightly.

During the course of cleavage, greatly variable individual gaps appear between the cells with white yolk or between these cells and those having brown yolk. In embryos in a more advanced cleavage stage, the surface cells form a compact mantle, whereby the inner cells containing white yolk are often a quite loosely arranged mass. The origin of the cleavage cavities to be discussed, which also are quite variable, is to be traced to these very variable gaps within the embryo.

In all the embryos shown, the extent of the pigmented cortex of the /340
egg does not seem to change. The regions of the gray crescent and the animal field cannot be differentiated here, but a portion of the egg cortex which corresponds to the extent of the vegetal field remains unpigmented. Judging by these prepared sections, the pigmented portion of the egg cortex in the animal field, as in the vicinity of the gray crescent, is not affected by the rearrangement but remains fixed. The same may be said of the unpigmented cortex of the vegetal field.

Wetzel emphasized that a thin surface layer of the egg does not participate in the yolk rearrangement process. When a white streak is formed, a portion of the vegetal field of the embryo surface becomes lighter in color, where it previously was dark. Furthermore, we shall describe the development of many embryos in which the sunken white yolk is visible as spots (frequently two spots) on the animal side in the

surface aspect. The white yolk cannot therefore be covered any longer at these spots by the completely opaque, pigmented cortex of the egg. We did not investigate just what happened to this pigmented cortex in this case, but we will return to it.

The white coloration of the vegetal field disappears in the experimental material, often with the exception of a few spots, and forms a spotted area which is colored with several hues of brown. According to these pictures and to all the other embryos examined, this is not based on the fact that pigment originates in the original cortex of the vegetal field, nor can it obviously be caused by a shifting of the pigmented cortical zone into this region. The partial or complete darkening of the vegetal field is caused by the fact that the brown yolk, which is forced upward, tends to more or less cover the sunken white yolk matter and underlies the white layer which remains. This is definitely recognizable in the meridianal sections presented here.

Finally, the question of exactly how the white yolk sinks downward must be discussed. Does this occur along a single plane of cleavage or at least principally along such a plane, in such a manner that the white yolk forms a sort of partition through the entire embryo with the brown yolk forming a separate complex on either side? Or does the white yolk move through the segmented embryo after rearrangement in the form of an irregularly outlined column from the vegetal to the animal pole without dividing the darkly pigmented matter into two parts? /341

Schultze noted only that the sections of eggs rotated in different stages of cleavage. The white yolk could be seen collected in the center of the egg. Wetzel described this by stating that the white yolk actually does seem to form a separation wall (see page 7). According to him, the yolk collects in the center as a result of rotation, and then extends itself further in the direction of the first furrow than towards the other furrow, thereby coming closer to the surface on one side. One therefore finds a sort of "actually very irregular separation wall" of large, white yolk cells in the direction of the first furrow through the blastula. The smaller brown cells lie to either side of this wall and are therefore separated by it.

Although Wetzel does state that the light yolk forms a very irregular partition, he emphasizes the fact that it does separate the dark parts into two halves, which are principally located on the right and left of the rotated embryo. Wetzel only studied these conditions on meridianal sections, so that his information could not produce any definite answers to the question at hand. The pictures discussed by us thus far are also meridianal sections of stages of cleavage. They are also not suited to the solution of the question. The question may only be solved if studies of latitudinal sections are made. One such section (Figure 4f) clearly shows that the white yolk does not sink principally

along a single plane of cleavage and cannot, therefore, form a complete separation wall which divides the egg in half during the cleavage. In the latitudinal section, the white yolk is presented as a round or irregularly outlined region.

Nevertheless, in the illustrated sections are seen jagged projections of the white yolk corresponding to the meridianal boundaries of the cell. Therefore, it can be conjectured that the yolk flows downward along such cell boundaries; however, it is now significant that this flow is not along one specific plane of cleavage (the first, as Wetzel indicates, the first or the second, possibly also the third, as we assumed prior to our investigation of these relationships in the section studies), but is along all perpendicular planes of cleavage about the egg axis. Figure 4f does not show that there is any preference for the two first planes of cleavage with respect to the spreading out of the white yolk.

We divided a total of 24 embryos from different females of the 1925 /342
and 1926 spawning periods into latitudinal section series, covering the period from the 4-cell to the morula stage. We did not find even a suggestion of a partition of white yolk in a single one of these sections, either in those eggs developing at room temperature after rotation or in those in which cleavage proceeded slowly because the eggs had been placed in ice water after rotation. It will not be necessary to supply any further examples of the cross section form of the white yolk. The picture is always similar to the one just presented.

The cleavage stages are not as favorable a time for the recognition of changes of yolk material arrangement as are the later stages of development (blastulae) in which the white yolk is distributed in large cells and the brown yolk in small cells. In most latitudinal blastula sections, the white yolk is arranged in exactly the manner given for the arrangement in the cleavage stages. One series of sections made through the region just under the vegetal surface, however, shows only one white streak in the direction of one of the furrows. We will return to this in our discussion of the structure of the blastula and gastrula.

We stated above that the white yolk in many embryos may be seen as white places on the surface of the animal side. Occasionally, such a white, or merely lighter, place may be observed in the middle of the animal surface. It is more usual that two such places are visible on this surface. In such cases they are opposite each other along the edge of the animal side and are, in reality, the ends of the white band across the vegetal surface (Schultze, Wetzel) that protrude over the edges of

the animal surface on both sides (see for example Figures 13a and b[1]). In such cases the white yolk cannot only have sunk into the interior of the egg, but its sliding must also have occurred over the outside surfaces

of the egg at two opposite points. The descent of a portion of the white yolk along the surface is shown in Figure 4b.

We mentioned that the brown yolk rises along the axis of the cells, which results in the fact that the white yolk in the middle of the egg and on its surface must ravel downward. Where a white band is present, it must lie in the direction of that one of the first two furrows, which was formed during the main time of descent of the white yolk. In cases where the ends of this band protrude onto the animal side, the white yolk, which descended on the surface, must have sunk along one plane of cleavage. This may be understood in the following manner. The furrow /343
first cuts into the animal pole, which lies at the bottom. After the passage of some time, its two ends, which always tend to gape apart, reach across the equator into the region of white yolk. This occurs at a time when the formation of cellular boundaries in the inside of the egg has not progressed very far. This probably creates the conditions which allow the white yolk in the vicinity of the furrow ends to sink along the cellular boundaries, which do not penetrate very far into the egg at this time, or actually along the surface of the egg in the direction of the furrow.

3. Summary

The first major determination to be made is that the white yolk in rotated eggs does not sink only along the first furrow, but rather along all meridianal planes of cleavage about the axis of cleavage, which are already present during the sinking process. As a result of this, Wetzel's statement that the white yolk forms a separation wall through the entire egg in the direction of the first furrow between brown yolk complexes lying to its right and left during the morula stage is false.

Second, the white yolk may also descend along the surface of the egg and, in such cases, also sink simultaneously along one of the first two furrows. The surface of the egg appears to be white in these places, although they lie within the darkly pigmented animal half. Furthermore, the white yolk sinking into the center of the egg may reach the animal surface and cause the appearance of a white, or light-colored, spot.

Third, these section pictures of cleavage stages show clearly that it is out of the question that, as a result of yolk rearrangement, each of the first two blastomeres attains the organization of an entire egg. Since the yolk does not divide the egg in the form of a separation wall in the 2-cell stage or in later stages of development, the 2-cell stage does not consist of two blastomeres, whose white yolk portions are turned toward each other and whose brown yolk portions are turned away from each other. The view of Roux, Wetzel and other authors, who state that each

of the first two blastomeres receives the organization of a whole egg and that they become independent of one another during the further course of development, and that duplication is to be attributed to this, is refuted by our investigation of cleavage in the rotated egg. /344

The question as to whether or not the region and the material of the gray crescent are affected by yolk rearrangement was not cleared up by our experiments. The fact that places on the dark cortex of the egg appear to be lighter or even completely white after the rearrangement of the yolk may only be understood by using the assumption that the darkly pigmented cortex disappears; however, the question to be answered is how this cortex disappears. Our experience has shown that morphological examinations to determine this would be rather difficult to conduct successfully. It is possible that progress could be made by vital staining of this cortex. We feel that the following two possibilities are the most probable.

It is possible that the entire surface layer of the egg is torn away by the rearrangement process, allowing the white yolk to appear directly on the surface. This is quite improbable since it would cause an extra ovate to be formed from the white yolk. The other possibility is that dark places remain on the cortex of the egg in spite of previous color lightening. This would allow for the assumption that an extremely thin and unpigmented surface layer of egg plasma did not displace during the rearrangement process.

Weigmann (1927) made detailed studies of the cortical layer of the egg of Rana fusca and determined that no plasma layer overlaid the pigment. In spite of this, such a layer might still be present in the living egg. During the formation of the gray crescent, Weigmann observed stripes, which indicate a flowing of the uppermost layer. We feel that this unresolved question will be answered positively in the results of the Schultze experiment, and we shall return to it.

PART III. THE BLASTULA

Schultze did not make sectional studies of the gastrulation of his duplications. Wetzel tried to plug this gap, thereby arriving at several important conclusions. He was not able to explain the process of gastrulation completely, however, and confined himself, as we have mentioned, to the examination of one form of duplication. A new and complete examination of the gastrulation process was therefore necessary. For this purpose, it was necessary to determine the peculiarities of the structure of the blastula stage in the rotated embryo. As could be expected from the behavior of the cleavage stages, there are three major peculiarities. /345

First, the arrangement of light and dark regions on the surface is changed. Second, the distribution of white and brown yolk is completely different from that in a normal egg. Third, the blastocele shows various individual formations. The descriptions of these peculiarities by Schultze and especially by Wetzel are reported on in the historical section of this study. In Part II we discussed the changes in the superficial aspect and the arrangement of the yolk substances in the later stages of cleavage. At this point, we would like to discuss the behavior of the blastula with respect to surface and yolk arrangement and the formation of its blastocele. To limit the number of pictures necessary for this purpose, we shall use stages of gastrulation in our discussion.

1. The Number of Cleavage Cavities

Table 2 presents information on the number of cleavage cavities in the blastulae and gastrulae sections examined. This information shows that not only two cleavage cavities may be present as Wetzel stated, but more--as many as three, four and even five. It is also possible that only one, or even none, may be present. Those cases in which only one cleavage cavity is present are in the majority by far. We observed 91 /346 embryos with one cleavage cavity and only 28 with two cleavage cavities; a ratio of about 3:1 (or 3.25:1 more exactly).

To evaluate this result more accurately we had to use the results of Table 1 as a comparison. These results show that from 237 (129 analyzed and 108 unclear) embryos, which survived long enough to yield an evaluative result, 166 = 86 + 80 developed into a clear duplication. Since, according to Wetzel, the development of duplication is the result

Table 2. Number of Cleavage Cavities in the Following:

1. Blastulae from female:								2. Gastrolae from female:							
Number	0	1	2	3	4	5	Total	Too old	0	1	2	3	4	5	Total
VII	-	4	-	-	-	-	4	-	-	13	-	-	-	-	13
VIII	-	8	1	1	-	-	10	-	-	22	2	-	-	-	24
IX	-	--	-	-	-	-	--	3	-	7	6	4	-	-	20
X	-	--	-	-	-	-	--	2	-	4	4	-	-	-	10
XI	-	--	-	-	-	-	--	-	-	2	2	-	-	-	4
XII	1	5	2	-	1	-	9	-	2	5	4	-	-	-	11
XIII	-	--	-	-	-	-	--	-	-	2	4	2	1	2	11
XIV	-	6	-	-	-	-	6	-	-	--	-	-	-	-	--
XV	2	3	-	-	-	-	5	-	-	--	-	-	-	-	--
XVI	-	3	1	1	-	-	5	-	-	--	-	-	-	-	--
5(1925)	-	7	2	-	-	-	9	-	-	--	-	-	-	-	--
Total	3	36	6	2	1	0	48	5	2	55	22	6	1	2	93
				Blastulae. . .				-	3	36	6	2	1	0	48
				Total.				5	5	91	28	8	2	2	141

of the presence of two cleavage cavities, the ratio of 3:1 taken from Table 2 should have allowed us to expect many less such duplications than we actually obtained. A maximum of one-fourth of the 237 embryos, or 60, would have developed into duplications. The remainder, approximately 180, would have yielded other formations, principally unitary embryos. Actually, exactly the opposite occurred. We obtained 166 clear duplications and only 72 embryos of other types, most of which also belong at least genetically to the duplication group (about the number x).

A comparison of these two tables shows definitely that, of the 141 embryos fixed and sectioned as blastulae or gastrulae, at least 3/4 (according to the ratio 166 + x duplications to 71 - x embryos of other types), or at least 105, would have developed into duplications, whereby a maximum of 36 would have been left over for other formations. If we use Wetzel's opinion and assume that all 28 of the embryos with two cleavage cavities were such that would develop into duplications, then 77 other cases of duplications remain, none of which can be attributed to the existence of two cleavage cavities. We must add only one other important fact to this general determination: from females IX, X, and XI, both embryos cut during the gastrula stage and other embryos were observed individually and were examined for the development of duplications. /347
In these special cases also the number of duplications was not correlated

to the number of double blastulae characterized by two cleavage cavities in their ratio to the number of embryos having other numbers of cleavage cavities. (Table 3).

Table 3. Comparison of the Results of Tables 1 and 2 for Females IX, X and XI

Females	Certain duplications	Other embryos	2 cleavage cavities	Another number of cleavage cavities
IX	23	5	6	11
X	15	11	4	4
XI	8	2	2	2

2. The Arrangement of White and Dark Yolk According to Section Pictures (Plate VIII; Plate IX, Figures 28-32 and 35-37; Plate X, Figures 38 and 39)

As we have mentioned on a number of occasions, Wetzel stated that the white yolk forms a partition inside the embryo in the direction of the first plane of cleavage between two separate complexes of dark yolk. If Wetzel believes that these two separate complexes lie on the right and left sides of the embryo, he must have proceeded from the erroneous assumption that the first plane of cleavage and the yolk partition always follow a median course through the embryo.

If one cuts through the middle of a blastula or a gastrula in the beginning stage in a direction perpendicular to the white band there is obtained a picture with respect to material distribution and except for the cleavage cavities and the possible presence of a blastopore slit, that is very similar to those presented by Wetzel (1895) in his Figure 20 on Plate XXXIV (see our Figure 2). The same is shown, for example, by our Plate VIII, Figures 23, 24, 25a and Plate IX, Figures 29, 32 and 37b. In these six examples we have, in the reproduced section illustrations, a mass of large white yolk cells extending from the vegetal to the animal side in the middle of the section, and on either side a complex of small brown yolk cells. The white yolks and dark portions are rather sharply differentiated.

More often it is found, in such sections oriented perpendicularly to the white streak, that the white yolk partition which, as will be shown, is only apparent, does not extend as far down as the animal side. Brown yolk usually is present on the animal side. The white yolk only descends to the animal surface in most infrequent cases, when cleavage has not been

retarded. A pronounced descent of the largest mass of white yolk into the animal embryo half occurs, on the other hand, in most cases in which the eggs are cooled after pressing and rotation (Plate VIII, Figure 27 and Plate IX, Figure 28). This difference has already been discussed (page 33). Even in those instances where the brown yolk forms a sort of cap in the animal region, it may appear at first glance that this brown matter is arranged on both sides of a white yolk partition. /348

If one tries to read the distribution of white and brown yolk only from individual sections which were cut perpendicular to the white band (or more generally expressed, parallel to a meridian of the egg), it is easy to be led astray, as was the case with Wetzel. This may be avoided by considering an entire series of such sections taken from one and the same embryo. We shall give two examples of this.

The section in Figure 25a (Plate VIII) shows, as we have mentioned, a defined white yolk partition. In other sections taken from the same embryo (XII 4)(Figures 25b and c), this partition does not reach the animal side, and the white yolk is encountered in the arrangement of section 25a in only approximately 12 sections. In embryo XIII 5, a middle section (Figure 37b Plate IX) shows a similar picture of an apparent partition of white yolk, whereas the yolk appears as an irregularly bounded mass in the interior in the other two sections (Figure 37 a and c). In an entire series of other pictures, the distribution of the white yolk is in the center and it is surrounded on all sides by a mantle of brown yolk (Plate VIII, Figures 20-22; Plate IX, Figures 30, 31, 35, 36; Plate X, Figures 38, 39a and b).

The same illusory picture of a yolk partition may also be observed sometimes in sections which were cut through an embryo without a white band parallel to a meridian. Examples of this are furnished by Embryo XIII 5 (Section in Plate IX, Figure 37b, surface view in Figure 26) and Embryo XVI 2 (Section in Plate VIII, Figure 23, surface view in Plate VII, Figure 12). In such cases one cannot assume from the superficial aspect (irregular roundish light field) that a yolk partition is present for the same reasons mentioned which led Wetzel to assume the existence of such a partition in embryos having a light streak; this is true in spite of the fact that according to many sections it appears that such a structure is present.

The question as to whether or not the white yolk actually forms the partition mentioned by Wetzel can only be decided, as was the case with the stages of cleavage, from sections oriented perpendicular to the axis of the egg. Embryo VIII 1 was divided into 88 such latitudinal sections. The series in the direction from animal to vegetal is in Figure 26c (Plate VIII), section 23; Figure 26b, section 35; and Figure 26a, section 46. The latter section (Figure 26a) was cut directly across the middle through the blastula. The embryo had a fairly good white band with a

very white center strip on the vegetal side at the time of fixation. The band extended dorsally somewhat onto the animal side. The last-mentioned section showed that the white yolk was more widely spread in the direction of the white band than could be seen on the other sections. The white yolk matter extended in this direction very widely towards the surface on one end. Absolutely no pigment was found at the surface at this particular spot. It is obvious that the area is the dorsal edge where the white band twisted around onto the animal side.

The extent of the white yolk is far less at the opposite end. Superficially, it is bounded there by a thick pigment layer. This is the area of the ventral end of the band. To the right, the white band in this section is rather regular in outline, while it is very irregular on the left; it extends with a rather thick projection deep into the left zone of darker matter. One still has the impression that this section shows two areas of dark yolk separated by a central zone of white yolk, in which the cleavage cavity is enclosed. This impression becomes even stronger when one views those sections lying closer to the vegetal side. If, on the other hand, one begins to view the sections lying more to the animal side, this impression that the yolk forms a partition between two complexes of dark material gradually is erased. The two sections from this area, which are shown (Figure 26b and c), indicate this very clearly. In a case such as this, in which the white yolk seems to form a partition also according to latitudinal sections, such observations of the yolk arrangement may only be made on the vegetal side of the embryo.

We must also particularly remark that we made a total of 14 latitudinal section series of blastulae and gastrulae, and the embryo from which the above-mentioned sections originated showed by far the best partition-like arrangement of the white yolk. We did determine in a number of embryos that, in some sections cut just under the vegetal surface, the white yolk was more spread out in one direction.

As we have shown, the white yolk does not form a continuous partition between two complexes of brown yolk either in the blastula or during the stages of cleavage. The only case among 38 (14 blastulae and gastrulae + 24 cleavage stages) in which the definitive latitudinal section showed a partition type of white yolk arrangement cannot be considered valid for an evaluation of matter arrangement in the rotated embryo. /350

We did not conduct more exact investigations concerning the appearance of the light-colored band or the light-colored region on the sections. It could not be expected that these would show more than a surface picture; namely, the presence of more or less large particles of white yolk on the corresponding surface locations. Such experiments could not, in all probability, yield any new conclusions on the question presented above (page 39) of whether or not an outermost layer of egg plasma was affected by the yolk rearrangements.

3. The Surface Picture of the Blastula (Plate VI and Plate VII, Figures 2-13)

a. The Form and Coloring of the Light Zone of the Vegetal Field

We have referred to the information furnished by Schultze and Wetzel concerning the surface aspect of the rotated embryos in Part I(A). According to them, there exist embryos having a white or light-colored band stretching across their vegetal surfaces, and others having an irregularly bounded white or light-colored field instead of these bands. In Parts IV and V of this study, we have furnished a large number of pictures which show the form and location of this white band or zone and which give further information concerning the very white areas, which may be differentiated within them[1]. We would like to remind the reader that the white bands or zones in these sketches are bounded by dashed lines and the lighter-white areas are outlined by dotted lines. In this section, we shall confine ourselves to a discussion of the surface aspects of the embryos photographed.

We should first like to refer to Table 4, in which the location of the first furrow and the white band relative to the median plane of the egg are given. The vegetal surfaces of the 12 embryos used in the compilation of this table are shown in Figures 2-13 (Plates VI and VII) in their blastula stages. No white band was present on the embryo presented last in Table 4 (Figure 2i), but rather an irregular, grayish-white vegetal field. In 5 embryos (indicated by *) we observed a beautiful white band (Plate VI, Figures 3e, 4a, 5, 6, 7a). In four others, the band-like character of the lighter-colored surface areas is very clearly distinguishable (Plate VI, Figures 8, 9a, 10a; and Plate VII, Figure 11a), but not so typically as it was in the first five mentioned.

Finally, in two other embryos, one might speak only of a band-like /351
arrangement of the lighter areas on the surface insofar as they show a somewhat larger extent in an oblique direction than they do in a perpendicular direction (Plate VII, Figures 12, 13a). Table 4 unquestionably allows for the conclusion that the white band may form any angle with the direction of the median plane. It may lie median, frontal or oblique to it, independent in every case of the direction of the first furrow (compare the first nine cases in Table 6 with one another). At the end of this section, we shall return to this point in more detail.

The typical white band observed in five cases is composed of two types of light-colored components. We see, namely, a very white middle

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[1]Part V was not published with Parts I-IV in Zeitschrift für Wissenschaftliche Zoologie, Vol. 130, 1928.

Table 4

Number of embryos and the plate pictures		Location of the band	Location of the first furrow
*13(25)	4a and b	median	median
20(25)	10a to c	median	median
*14(25)	3	median	frontal
*17(25)	7a and b	median	frontal
18(25)	8	median	frontal
21(25)	11a and b	frontal	frontal
*16(25)	5	frontal	median
⊥ *15(25)	6	oblique	oblique
19(25)	9a and b	oblique	frontal
11(25)	13a and b	frontal (band-like)	oblique
XVI 2	12	oblique (band-like)	median
XVI 1	2i	no band	median

* = Embryos with a beautiful white band.
⊥ = The directions of the band and the first furrow are perpendicular to one another.

strip bordered on both sides by grayish-white material. The relative quantitative ratio, the form, and the extent of the two components vary somewhat in each embryo, as could be expected. We should like to consider the model type to be a band, the light white stripe of which extends, as in Figures 4a and 5, just about through the middle of the vegetal surface from one edge to the other: median, frontal, or oblique.

The grayish-white component borders the stripe on both sides in varying widths. A slight deviation from this type results when the white band extends across somewhat onto the animal surface on one end (Plate VI, Figures 3e, 6, 7a). In many instances, this end is wider /352
than the other, and the position relative to the median plane plays no part in this respect. In Figure 7a, the band crosses over onto the animal surface exactly median to the dorsal edge. This occurred in Figure 6 on the left ventral edge. In other cases, this varied. As we shall discuss later, it is more usual for both ends of the band to reach over onto the animal surface. The very light portions of the white band may also be present to a less noticeable degree, i.e., having smaller dimensions.

In four other cases (Plate VI, Figures 8, 9a; Plate VII, Figure 11a; Plate VI, Figure 10a) we see, for example, that these lighter portions gradually decrease from the first to the fourth case. In the latter, they

contrast only slightly to the less light portions. In spite of this, one may still refer to a clear white band in the vegetal surface with a lighter middle strip and darker outside edges. This differentiation between very white and less white portions on the vegetal surface may in general always be made, even when no pronounced band is present; for example, in Embryo XVI 2 (Figure 12, Plate VII). In this instance we also see a middle strip of very white material surrounded by gray-white. The whole area is irregularly bounded, but may still be considered to be somewhat band-like. In a second example, there can also hardly be any question of it (Plate VI, Figure 2i). One very often finds cases in which the lighter material as a whole does form an irregularly bounded, roundish complex on the vegetal surface, but these very white portions often appear to be extended definitely in the direction of one of the first planes of cleavage. An example of this is given in Figure 6a. Furthermore, there are enough illustrative sketches in the sections following this one.

These few examples serve to illustrate sufficiently the fact that instead of the original round vegetal white field on the vegetal half of the rotated embryo in the blastula stage, a lighter zone appears, which may be formed in a great number of different ways. One can, however, almost always differentiate a more central region of very white matter on the vegetal surface holding apart gray-white edge portions. The embryos may behave differently with respect to this last point in that the very white areas need not always lie in the middle of the surface. They may also be present in more than one place. We see, for example, in the case of Figure 13a (Plate VII), that the lighter material as a whole give the impression of a band on the vegetal surface, but /353
that rather dark material is found on it in the middle of the vegetal surface, while a very white complex is present at each end of the band.

b. The Location of the White Band

A few further considerations concerning the location of the white band, insofar as the light zone has the form of such a band, may be gained through a study of the data contained in Tables 5 and 6. Table 5 is a compilation of the aspect of the vegetal surfaces of embryos which developed at room temperature after pressing and rotation. The second furrow appeared, therefore, in these embryos soon after rotation. This furrow was formed principally simultaneously with the rearrangement of the egg substances. This table shows that, of 230 embryos having a white band, this band followed a course definitely perpendicular to the direction of the first furrow in 181 cases. This means that it lay in the direction of the second furrow, which generally is perpendicular to the first furrow. The direction of the band coincided with that of the first furrow in only seven instances.

Table 5. Cleavage at Room Temperature

Direction of first furrow	No band	Band present			Total
		⊥ to first furrow	∥ to first furrow	Oblique to first furrow	
Frontal	17	59	1	3	80
Median	42	66	2	12	122
Oblique	57	56	4	27	144
Total	116	181	7	42	346
		Total 230			

Table 6. Cleavage in an Icebox

Direction of first furrow	No band	Band present			Total
		⊥ to first furrow	∥ to first furrow	Oblique to first furrow	
Frontal	165	12	16	4	197
Median	113	17	16	1	147
Oblique	123	6	2	8	139
Total	401	35	34	13	483
		Total 82			

It could not be determined without specially designed studies just how many of the 42 bands, which were oblique to the direction of the first furrow, had a direction coincidental to that of the second furrow. We neglected to determine whether or not and to what degree the embryos having a frontal and median first furrow had the second furrow oblique to the first. If such cases were contained in our series, it is entirely possible that an oblique position of the white band coincides with the direction of the second furrow. We can disregard such cases however.

In spite of this, 181 bands were perpendicular to the direction of the first furrow and therefore probably all ran a course coincidental with that of the second furrow, while only seven cases had bands running in the direction of the first furrow; this is a ratio of 26:1. It was shown that, when the cleavage process was not disrupted by a time factor

(note: either retarded or accelerated) and in the event that a band develops through the material rearrangement, it is formed in the great majority of cases in the direction of that furrow, which cuts in during the main rearrangement process.

Table 6 evaluates only those embryos which were pressed and rotated during the stage of the incisive and completed first furrow and then placed in an icebox. The further course of cleavage was therefore strongly retarded in these embryos. Correspondingly, we see that the white band forms much more often in the direction of the first furrow. Of the 82 bands observed, only 35 lay perpendicular to the direction of the first furrow, whereas the direction of 34 coincided with that of /354
the first furrow. If we disregard once again the 13 bands which were oblique to the first furrow, we have with this cleavage retardation a ratio of 1:1, which corresponds to the ratio of 26:1 given above for normal conditions. This represents a tremendous shift of the location ratio of the white band. This corresponds to the other experimental conditions. The main rearrangement of the egg material occurred in these embryos before the first furrow was entirely completed.

In general, our determinations showed that the white band does not occur too often. In most cases the distribution of the light material on the vegetal surface shows only an irregular outline. A comparison of the results contained in these two tables could lead one to the conclusion that the retardation of cleavage (Table 6) sharply suppresses the formation of white bands. In Table 6 (retarded cleavage) we have 401 embryos without bands and 82 with bands: a ratio of 5:1. In Table 5 (cleavage not retarded), on the contrary, we have only 116 blastulae /355
without bands and 230 with bands: a ratio of 0.5:1. This correlation is, however, only apparent. It may be explained simply from the material which was compiled in these two tables.

In Table 6 we only considered the eggs from three females. Almost all of those which were pressed, rotated and marked are included in the data. The embryos in Table 5 are only selected individuals from a larger number of females in different experiments. They were chosen from the number of marked eggs so selected that the majority had a band. Table 5 represents a selection of embryos which were observed individually. We chose those with white bands for two reasons. First of all, the data furnished by earlier authors indicated that duplications were most often produced from such embryos. Secondly, and most important, the white band furnishes an important orientation marking for the evaluation of material shifting during gastrulation.

On the one hand, we determined, contrary to Wetzel, that the white yolk inside the blastula does not represent a continuous partition between two complexes of dark cells. Only in one exceptional case were we able to determine in the direction of one of the first planes of cleavage

a certain widening of the white yolk. This was observed, however, only in the vegetal half of the embryo in question. On the other hand, we agreed with Wetzel in our determination of the presence of the white band in a large number of instances. Wetzel considered this to be the vegetal edge layer of the partition in the interior of the embryo. According to our determination that no partition exists, this opinion of Wetzel's cannot be valid.

Wetzel also stated that the white band only occurred in the direction of the first furrow. Since, as we have said, he felt that the white yolk formed a partition inside the egg and that it also lies in the direction of the first furrow, his determination of the course of the white band in this direction is easily understandable. He imagined that, after rotation in the two-cell stage, the white yolk sank along the first plane of cleavage and achieved a greater spread in this direction than in any other. This led him to the conclusion that the white band (the edge of this partition) must also run in this direction. As we have demonstrated above, the white yolk does not sink only along the first furrow, but rather along all eight planes of cleavage which occur around the cleavage axis. No partition inside the embryo with a favored meridianal longitudinal spread therefore is formed. /356

In spite of this, the white band appears on the surface. Its presence cannot be caused by the arrangement of the white yolk within the embryo. Furthermore, we observed that the white band occurred not only in the direction of the first furrow, but also in the direction of the second. Our more exact examination showed the result that the white band, when such is formed at all, lies in the direction of that furrow which cuts through the embryo during the period when the main rearrangement processes are going on in the vegetal region. Our explanation for this will be furnished later in this study.

4. The Position, Form and Size of the Cleavage Cavities (Plates VIII-XI)

According to Wetzel, a small cleavage cavity should be present on both sides of a yolk partition in the interior of the embryo. They lie in the vegetal region of the embryo in the section which he presents as a demonstration of this assumption (Figure 2). We also observed such pictures, but only in rare exceptions (Plate IX, Figure 36) and usually only in a few sections from a series, whereas other sections showed other cavities which were often located in entirely different places. This may be illustrated by a comparison of the section from Plate VIII, Figure 25a with that in Figure 25b and c from the same series, the sections in Plate IX, Figure 37a-c, and the two sections from Plate X, Figure 39a and b, which also came from the same series.

As we have briefly mentioned above, we also encountered cases with no cleavage cavities (see Table 2). In such blastulae the white yolk could be either loosely arranged or very tightly packed (Plate IX, Figure 29).

As may be determined in Table 2, only one cleavage cavity is usually present, which may often be very large in contrast to the generally small cavity of a normal embryo (Plate X, Figures 45, 46, 48; and Plate XI, Figure 50). Its form is very irregular and its position relative to the egg material varies greatly.

Plate VIII, Figure 20, shows the yolk in a very loose form in the vegetal region, and here also a type of cleavage cavity is formed. The loose arrangement of these yolk cells is characteristic of many embryos. In Plate VIII, Figure 21, a cleavage cavity is also recognizable in /357
the vegetal zone in the midst of dense white yolk. This cavity is longitudinally somewhat extended in the vegetal-animal direction. In Figure 22 (Plate VIII) we see a reversed extension of the cleavage cavity. In this picture it is located in the vegetal area of the white cell mass and transverse to it. The cleavage cavity usually has a very pronounced vegetal location in those embryos in which cleavage has been retarded through cooling (Plate IX, Figure 28). In the embryo shown in Figure 23 (Plate VIII), the cleavage cavity is located in the white yolk in the animal region and is composed of a very branched system of hollow spaces.

A cleavage cavity also very near the animal region is found in Figure 32 (Plate IX). It has an oblique position in the white yolk. A picture, which is seen relatively often, is presented in Figure 24 (Plate VIII). It shows a rather large cleavage cavity, which stretches in length from the vegetal towards the animal side, through the middle of the white yolk. The same may be seen in Figure 33 (Plate IX) and Figure 42 (Plate X). In other instances, the one cleavage cavity present is not in the white yolk itself, but rather completely on one side, and provides a more or less sharp border between the white and dark material found there (Plate IX, Figures 30, 31; Plate X, Figures 45, 46; Plate XI, Figures 49, 50).

Even when two cleavage cavities are present, their size and location change considerably. In Figure 36 (Plate IX) the location of the relatively small cleavage cavities corresponds approximately to the data furnished by Wetzel (see Figure 2). In Figure 34 (Plate IX), this is not the case. First of all, the cleavage cavities in this case are very large, especially one of them. Furthermore, only the latter is on one side of the white yolk between dark and light material. The smaller cavity is in the middle of the white yolk material itself. The same is true for the embryos in Figure 35 (Plate IX) and Figure 41 (Plate X).

In another embryo with two cleavage cavities, which is not shown in this study, a very good white band was present. In the interior of the embryo the white and dark yolks were relatively sharply separated. Corresponding to the frontal position of the external white band in middle sections, it showed a slightly larger extension in the same direction. The two small cleavage cavities located in the vegetal portion of this embryo were not located dorsally and ventrally to this apparent partition, but rather in the middle of the white yolk itself, with one each located in the right and left region of this white material. Two cleavage cavities in a pronounced vegetal location, which correspond to Wetzel's statements, are shown in Plate VIII, Figure 27. The embryo shown here /358 was cooled during cleavage.

An example of three cleavage cavities is given in Figure 38 (Plate X). In this case a rather large cavity is located in the white yolk on the vegetal side. Another rather large cavity is located in the animal region, where it forms a border between the light and dark yolk. The third, very small cleavage cavity is located approximately between the other two and separates white and dark material on one side.

An example of four cleavage cavities is shown in Figure 37a-c (Plate IX). No cleavage cavity is shown in the sections cut through the middle of the blastula (Figure 37b). The cells on the edge of the white yolk complex lie rather loosely. This picture may only be seen, however, on five sections. When the series is followed further in either direction, two beautiful cleavage cavities appear (Figure 37a and c). They sharply separate the light and dark yolk material on the sides.

If one had only the series of these three sections for evaluation, the supposition might be made that the two cleavage cavities, which are both on the same side of the white yolk, were located in an area where no such cavities should be seen, and where only the white yolk cells on the edge of the entire complex are very loosely arranged, yet connected with one another at this location. This supposition is not, in reality, valid when the entire series of sections is evaluated. Actually, four completely separate cleavage cavities are located in this embryo. This was also true in a second case, where three vegetal and one animal cavities were found (Figure 25a-c, Plate VIII). When one goes through this series in a given direction, two of the vegetal cavities are found first (Figure 25a). These then disappear in order to give way to the animal cavity (Figure 25b), which is then replaced after its disappearance by the third vegetal cavity (Figure 25c).

The last case to be presented is one having five cleavage cavities (Plate X, Figure 39a and b). Here, we have two vegetal and three animal cavities, of which the last three separate the dark and light material rather sharply, whereas this is not particularly true of the two vegetal cavities. In general, it can be stated that a mantle of cleavage

cavities on this embryo separate the light yolk nucleus from the dark calotte to a certain extent. We should also mention that the sections shown lie 35 sections apart in the series.

5. Summary

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A blastula, which develops from a pressed and rotated egg, differs from a normal blastula in the following points.

First, the white yolk is more or less sunken into the animal half of the egg and forms an irregular mass in the interior of the egg, which is not at all a partition in Wetzel's sense. Sometimes, just under the surface of the vegetal region, white yolk appears in the form of stripes in the direction of one of the first furrows. The brown yolk has more or less ascended into the vegetal half of the egg and underlies a varyingly large portion of the cortex of the vegetal side of the embryo.

Second, such blastulae show a large degree of variability in the formation of the cleavage cavity. Usually only one is present, or it is completely lacking, or as many as three, four, or five may be observed. The form, size and location of these cavities vary just as their number does.

Third, the surface aspect is very noticeably changed. In place of the normal vegetal field, one sees a zone of less white and light white material on the vegetal side of the blastula. This material either appears as a typical white band or, associated with this conformation throughout all the transitions, a region of quite irregular outline, whereby the distribution of the very white areas varies greatly. The light white areas obviously appear because the white yolk lies directly under the hypothetical outermost plasma layer. Either the white yolk, most of which has sunk into the interior of the egg, extends outward to the surface, or larger or smaller white yolk complexes have remained suspended while their major mass has descended. When one or both ends of a white band reaches across the edge into the animal field, the white coloring of these ends must be attributed to the white yolk which has slid along the surface of the egg and remained hanging there. The less white areas are those under which the ascended brown yolk lies.

A fourth point is, as we have already stated (page 39), as yet unexplained. The experiments concerning the rearrangement of the yolk did not explain the question of whether or not the region of the gray crescent is affected by this rearrangement of yolk substances. In the first case, the blastula developed from the rotated egg would be somehow different from a normal blastula with respect to the gray crescent,

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which is an especially important region from the development of physiology standpoint. In the second instance, just as is the case with a normal blastula, it would retain the region of the gray crescent in its original dimensions and location.

PART IV. GASTRULATION

Since the organization of the blastula in a rotated egg is changed, it follows that the gastrulation process, which begins after rotation, must also deviate from the norm. The fact that Wetzel's conceptions of this could not be accurate is already apparent from his idea that two cleavage cavities were always present and were a necessary condition for the formation of a duplication. We have already presented this information in a short article (Schleip and Penners, 1926). We shall now present a detailed account of the course of gastrulation in a rotated egg, first through sketches and photographs of the surface and then through the examination of section series taken during stages of gastrulation. The extreme diversity of gastrulation processes will also be presented in Part V, where we describe the development history of many embryos, starting at the beginning of gastrulation.

A. Superficial Aspect of Gastrulation (Figures 5-16 and Plates VI and VII)

The normal gastrulation of the embryo of Rana fusca is evidenced on the surface of the embryo in the following manner. A pigmented line forms dorsally on the boundary between the gray crescent and the vegetal field across the median. This line deepens into a sickle-formed, groove-like depression which is concave to the vegetal field. Its upper (or anterior) edge is designated as the dorsal blastopore lip. This blastopore lip becomes horseshoe-shaped and simultaneously bounds the vegetal field laterally by extending along both sides of the medians; the lateral blastopore lips originate in this manner. Finally the blastopore lip bounds the vegetal field ventrally also; the ventral blastopore lip thus originates and therewith the blastopore groove closes to a circle about the vegetal field.

Already during this process the dorsal blastopore lip extends toward the vegetal pole, whereby a corresponding portion of the vegetal field disappears under it. When the blastopore groove has taken on a circular shape, the entire circular lip shrinks together almost concentrically, whereby, however, the dorsal edge continues to grow more rapidly. This causes that portion of the vegetal field seen from the outside to become ever smaller, so that one first speaks of a middle sized, and then of a small yolk plug. If the blastopore has closed to a small split, the white yolk material has then disappeared into the interior of the embryo. /361

According to the experiments of Vogt (1922 a-c) and Mangold (1923), three partial processes are to be distinguished in the gastrulation of the Triton embryo. These are unquestionably also found in Rana. The white yolk of the vegetal field has descended into the interior of the embryo in a manner not further discussed here. The marginal zone, which corresponds to the region of the gray crescent in Rana fusca, is tucked in around the blastopore lips into the interior. Through the surface extension of both the animal field and the marginal zone, the epibolic growing over of the descending white yolk is effected.

As Wetzel proved, the occurrence of a circular groove on a rotated egg, which was also observed by Schultze, is the external evidence of a double gastrulation. The description of the formation of this groove, however, is only an example of many different forms. In a rotated embryo, one can find every conceivable type of transition stage between an apparently normal blastopore and Wetzel's groove.

In our description of the external course of gastrulation, we shall partially refer to pictures and descriptions found in Part IV, Section B. This allowed us to confine the number of pictures necessary for this part of the study within reasonable limits. The photographs referred to are surface shots of embryos showing the gastrulation in sections. Furthermore, the description given in this section will be supplemented in Part V.

1. The Formation of a Seemingly Normal Blastopore

A characteristic example of how a seemingly normal blastopore may form on a rotated egg is furnished by a description of Embryo II 24. Corresponding to the median position of the first furrow on the vegetal field, it had a lighter field which was strongly lengthened in a frontal direction and had to be described as a rather clear band (Figure 5a). Within this band is observed a very white area which was similarly lengthened and frontally directed. The dorsal edge of this area coincided with that of the very light field. On the border between them, we observed a short, frontally placed, ventrally slightly concave, brown pigmented furrow, from which irregularly branched brown pigmented lines emanated laterally. The picture was therefore very similar to one of an embryo having a normal blastopore anlage just appearing. (Figure 7a gives a more detailed sketch of a quite similar blastopore anlage.) The cells within the lighter field were always recognizable and were arranged in general lines in the direction of the arrow (Figure 5a). This is obvious evidence that the cell material of the lighter field is shifting in these directions; i.e., toward the furrow. /362

These cells extended along the entire dorsal edge of the light white area, whereby their own dorsal edge, in reality the dorsal

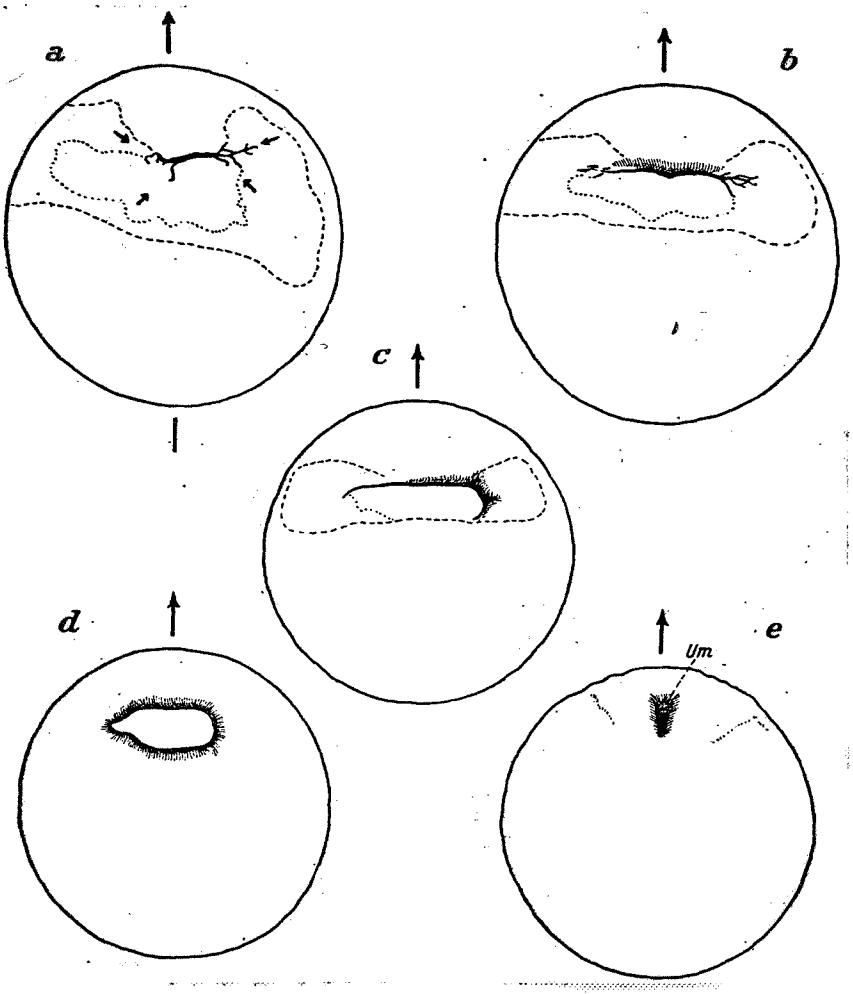

Figure 5. Embryo II 24. a, March 12 at 1:45 p.m.; b, at 7:45 p.m.; c, March 13 at 12:15 a.m.; d, at 4:45 a.m.; e, at 4:45 p.m. Um, remainder of blastopore

blastopore lip, began to swell. The size of the lighter field, thereby, decreased clearly; this may be explained by the fact that the cell material penetrates into the interior by tucking in around both edges of the groove (Figure 5b). This is a deviation from the norm, where the tucking at this stage only occurs around the dorsal lip of the blastopore groove; i.e., around the dorsal blastopore lip. This deviation from the norm did not, as we shall see, remain in effect. The blastopore groove at first curved around the light white area on the right side (Figure 5c) and then encircled it (Figure 5d) so that it was surrounded like a typical yolk plug by an oval, not circular, blastopore lip. During this process the extension of the lighter area around the

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blastopore decreased further in size (Figure 5c) and finally disappeared completely (Figure 5d). This can only have been effected by the tucking-in of the edge of the blastopore, not around both edges of the groove as was the case at first, but rather only around the outer edge. In this respect, the blastopore was now completely comparable to a normal one. It then closed into a small, median split (Um in Figure 5e).

Even though this gastrulation process is very similar to the norm, it does depart from normal in two points. First, in the stage represented in Figure 5a (beginning of gastrulation) material also tucks in around the ventral edge into the interior. Second, the blastopore closes itself on the spot after it becomes oval and tucking occurs only around its outer edge. These two deviations are very significant, since they allow for the following conclusions.

First, the initial blastopore anlage does not border on material, which, in a normal egg, would be absolutely incapable of tucking (vegetal field). For a time, the white material in this embryo located ventral to the blastopore had the capability to tuck in around the ventral edge of the groove. Second, the surface extension, the precondition for the tucking process and for the epibolic overgrowing of the yolk plug, did not confine itself principally to the region between the first blastopore anlage and the original animal pole. On the contrary, the region of the original vegetal field must also have been capable of surface increase at this stage, at least partially. Expressed succintly, the difference between the dorsal and ventral blastopore lip must be slighter in this embryo than in a normal one. The described embryo produced a "unitary formation," which was not examined in detail (see Part V). In many other similar cases, certain duplications were produced.

Figure 23 shows another embryo as seen from the vegetal side. The /364
blastopore anlage of this embryo also seems to be almost normal at this stage. Figure 29 shows a further developed stage of another embryo. The blastopore lip here is already approximately circular, while its dorsal part is obviously more advanced in development. Both embryos are treated more exactly below. Embryo XIII 2, which is very similar to the first one, may be seen in Plate VII, Figure 19. A light white complex was located to the left on the vegetal surface of this embryo. On its dorsal border, a seemingly normal blastopore lip was formed. It soon surrounded the plug laterally (Figure 19a) and then became a blastopore which appeared to be completely normal. Only its location on the left side was abnormal. This leads to the conclusion that the location of the light white field has an effect on the location of the blastopore anlage in the rotated egg. Figure 19 shows a stage in which the blastopore is already much diminished and the yolk plug has sunk to a large degree. Very pronounced furrows (Figure 19b) may be seen on this embryo. Such furrows are normally never present. They will be described in detail in the discussion of other embryos.

Embryo XII 2 (Plate VII, Figure 18) belongs to the group whose blastopore formations seem to be rather normal. A rather long groove, perpendicular to the obliquely-oriented first furrow developed; in other words, the groove was also oblique with respect to the median and near the dorsal side and thus was in the region of the original gray crescent and also on the dorsal boundary of a light white complex on the vegetal surface (Figure 18a). A pigment line originated at this groove and ran through the light white complex. Then the entire light white complex, including the pigment line, was enveloped from the right by the groove (Figure 18b). It was then further enveloped from the left, so that a small, round yolk plug was formed which lay within a seemingly normal round blastopore (Figure 18c). The blastopore closed (as in the embryo in Figure 5e) on the spot (Plate VII, Figure 18d). Aside from the somewhat different location of the light and light white material and of the first anlage of the blastopore groove and the developed blastopore, this photographed embryo is very similar to the one described above in detail with the aid of sketches.

Summary. When a more extensive pure white area is located on the vegetal surface within the lighter zone on a blastula formed from a rotated egg, the first blastopore anlage is formed as a groove which lies more or less exactly on the dorsal edge of the light white material and therefore in the region of the original gray crescent. This groove is very similar to a normal start of a blastopore. In at least a number of cases, material on the surface first tucks around both the dorsal and ventral edges of this groove and into the interior of the embryo. Then however, the blastopore groove gradually curves around the entire light white complex, so that a relatively round, seemingly normal blastopore is formed, the edge of which clasps the very white material into a yolk plug and then closes over it. During this entire process, surface material continuously rolls over the blastopore edge into the interior of the egg. It seems that not much more material tucks in over the dorsal edge than over the ventral. This, as well as the closure of the blastopore on the spot, indicates further that the remainder of the surface in the entire area surrounding the blastopore is capable of surface increase in a similar manner.

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2. Formation of a Groove Running in a Straight Line, Which is Wide at First with a Large Yolk Plug

The type of blastopore formation previously described leads to the development of an approximately round blastopore. The form of gastrulation described in this and following sections leads to the formation of a blastopore having the form of a more or less straight groove with two lips. Such a blastopore form will be designated briefly as a groove.

a. Development from only one gastrulation anlage

The formation of such a groove can obviously be based entirely on the form of the very white material. Embryo X 11 (Figure 6) provides a good example of this. No pronounced light band was present on its vegetal surface. The lighter region of the vegetal surface had an irregular border, but was somewhat more extended in the direction of the median plane in which the first furrow was also located, and contained a strip of very white material running in the same direction (Figure 6a). Both the light region in general, and especially the very white material, came to an end with a wide edge dorsally approximately on the vegetal border of the region of the original gray crescent. Gastrulation began, therefore, here on the border between the original gray crescent and the white material by forming a short, frontally-placed, groove-like depression (Figure 6a). Therefore, in this stage the embryo resembled /366
a normal one in that this depression might represent the dorsal blastopore lip, which differed from the norm in that it was not curved. The further course of gastrulation deviated, however, very strongly from the norm.

The very white strip was enclosed from its dorsal end by the end of the groove (Figure 6b), which then extended itself in a ventral direction along this strip (Figure 6c), until it completely enclosed all the light material. Thus, a rather wide, approximately median groove was formed at first. The very white material was sunken between its lips in /367
the form of an oblong yolk plug, while the lips themselves began to approach each other. As may be seen easily, the lips were first applied against each other in the ventral region where the strip ended in a point and was at its narrowest. The groove then continued around the ventral edge for a short distance onto the animal side (Figure 6c[1]).

The groove, which was first wide, became thinner in the area of the vegetal surface and then closed, starting at the dorsal end (Figure 6d), until finally it could no longer be seen on this surface (Figure 6e). On the other hand, it lengthened more and more on the animal side of the embryo, almost surrounding it completely (Figure 6e[1]). It then closed continuously in the same direction on the animal surface (i.e., starting from the ventral edge also (Figure 6f[1]) until it completely disappeared on this side. The very white material was therefore sunken between the lips of the originally wide groove in the form of a yolk plug. The small amount of white material visible on both sides of the very white strip in Figures 6a and b also disappeared. It must have been tucked into the interior of the embryo around the right and left lips of the groove between the stages shown in Figure 6b and c.

The embryo with a narrow groove, which will be discussed later in this study, is similar to this one in that it became narrow after its initially wide groove folded its edges together over the yolk plug.

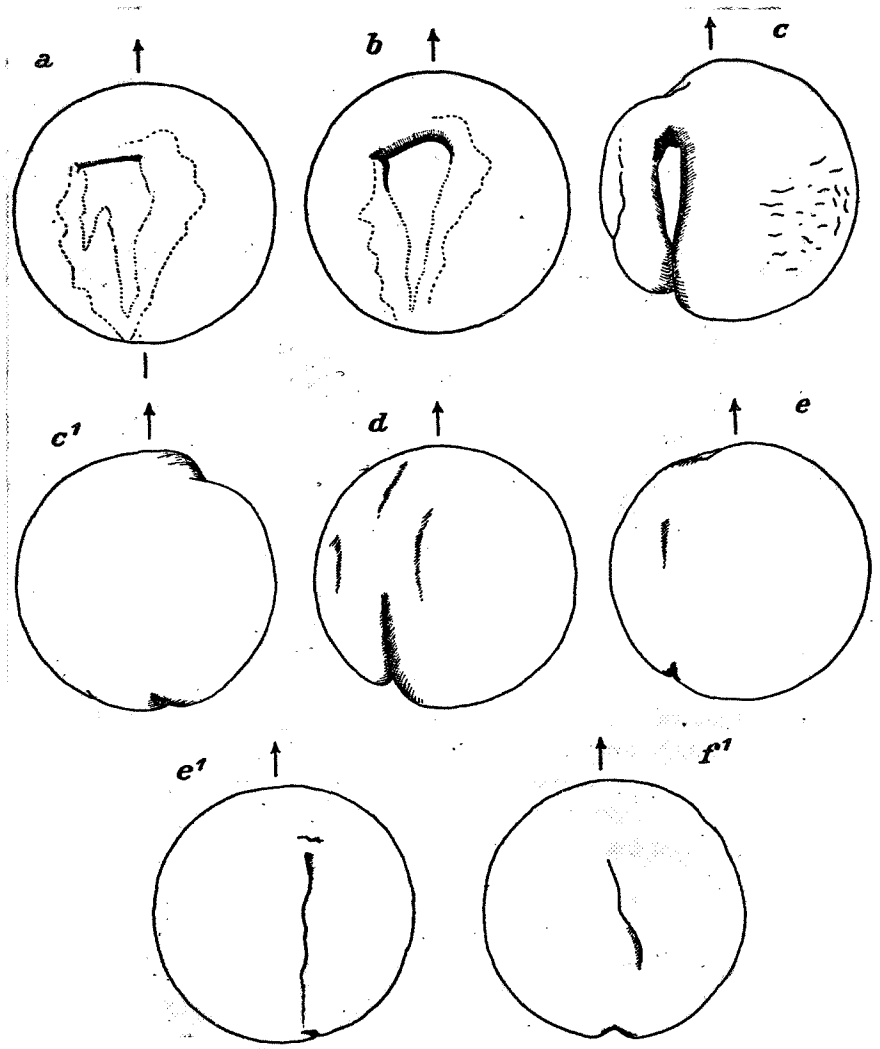

Figure 6. Embryo X 11. a, April 1 at 2:45 p.m.; b, at 8:30 p.m.; c and c^1, April 2 at 1:00 a.m.; d, at 6:30 a.m.; e and e^1, at 11:30 a.m.; f^1, at 6:00 p.m.

d, one furrow each was located on both sides of the closing groove

It is also similar in that the wide groove on the vegetal side extended itself around the ventral edge far onto the animal side, in the form of a very narrow slit. The embryo described should be included with that shown in Figure 31a and b, which will be discussed later.

Summary. A seemingly normal, first blastopore anlage was formed dorsally on the vegetal border of the region of the original gray crescent. Its

localization was apparently dependent upon that of the edge of the very white material. The first blastopore anlage, likewise determined manifestly by these contours, extended itself in such a manner that it formed two lips which enclosed the very white strip on the right and left. While light material was tucking-in over these lips, they grew toward each other across the long yolk plug so that the initially wide groove became very narrow and finally closed. Both the tucking-in and the closing of the blastopore lips are only conceivable when the principal surface enlargement of the remaining material proceeded in totally different directions than would normally be the case. This embryo is similar to the one with a narrow groove in that this wide groove extended itself around the ventral edge onto the animal surface and was in the form of a very narrow slit from the outset. /368

b. Development from two gastrulation anlagen

The normal circular blastopore lip is formed by the extension of a single anlage: the dorsal blastopore lip. This was also true in the development of the seemingly normal, roundish blastopore lip and of the initially wide groove of the rotated egg which has just been described. In a number of cases, but infrequently, we observed the occurrence of two initially separated blastopore anlagen.

Such phenomena are shown in Figures 21 and 22 and in Figures 20 and 30a. These are the surface pictures of embryos, to be discussed later in this section, which were cut. We do not know, therefore, just how the surface aspect developed in further stages. Only one of the embryos was fixed in the stage shown in Figure 30b. This embryo formed an initially wide groove. Judging from the form of the very white material, the embryo in Figure 20 might have developed in the same way. The very white zone in the embryos shown in Figures 21 and 22 were very wide in proportion to their lengths. It is very probable that the two separately occurring blastopore anlagen became elongated, enclosed all the very white material, and thus formed an approximately round, and therefore seemingly normal, blastopore. According to this, it is probable that such a blastopore could also be formed from two separated anlagen under certain conditions, and not only from the initially wide groove, which is being discussed here.

In all of these four embryos, the more dorsally located blastopore anlagen either originated earlier, or, which indicates the same thing, were more strongly developed. These dorsal anlagen are obviously dependent on the location of the edge of the very white material. Simultaneously, their locations indicate that they are dependent on the location of the original gray crescent. The dorsal blastopore anlage is located in a position, at least in Figures 21, 22 and 30a, corresponding to that of the vegetal edge of the gray crescent. The more ventrally

located anlage unquestionably did not develop dependent on the middle region of the original gray crescent, but rather only in relationship to the edge of the very white material. One may state this succinctly by saying that, in these embryos, the dorsal blastopore lip appeared first, followed by the ventral lip, which appeared before the formation of the lateral blastopore lips.

The embryo, described in detail in Figure 7 (Embryo I 18), corresponds very closely to those shown in Figures 20 and 30. Figure 7a shows its first gastrulation beginning which was very similar to that described for Figure 5a and which was mentioned in connection with it at that time. The major difference between Figures 5a and 7a is that the very white strip is shorter in 5a than it is in 7a. For this reason, the roundish blastopore forms in 5a, but an initially wide groove forms in Embryo I 18. In Figure 7a a streak of less light-colored material extends ventrally toward the formed blastopore groove and from the other, dorsal side quite dark cell material advances towards the groove. This is an indication that in this stage surface material is tucking-in around the ventral and dorsal edges of this still very slightly developed blastopore groove. /369

The groove located on the dorsal edge of the very white material extends pronouncedly along this edge in a frontal direction (Figure 7b and c) and then extends from right to left across almost the entire vegetal surface before the ventral border of the very white mass is enveloped by a groove (Figure 7d); as has been mentioned, an initially wide groove is formed. It is almost certain that this ventral groove originated independently of the dorsal groove, even though its appearance occurred fortuitously between two controls. Figure 7a-f shows the further course of the tucking-in of the very white material very well. After this had occurred around both the ventral and dorsal edges of the dorsal blastopore anlage, as we mentioned above, it was confined to the dorsal edge from Figure 7c onward. The tucking-in of ventral, light material around the ventral edge of the groove seemed to cease and then recurred more actively after the ventral groove had formed (Figure 7d).

At about this time, all the less light dorsal material with the exception of a small residue on the left side had tucked in around the dorsal edge. The less light material on the ventral side now disappeared by tucking-in around the ventral groove also with the exception of a small residue on the left side (Figures 7e and f). As was the case with Embryo X 11 (Figure 6), the two blastopore lips closed over the sinking yolk plug, in this case from both sides (Figure 7d-f). The closing of this now very narrow groove progressed from the right, so that the last remnant of the groove was visible on the left side.

Summary. An initially wide blastopore groove may be formed from two separately occurring blastopore anlagen. Both anlagen appear on the

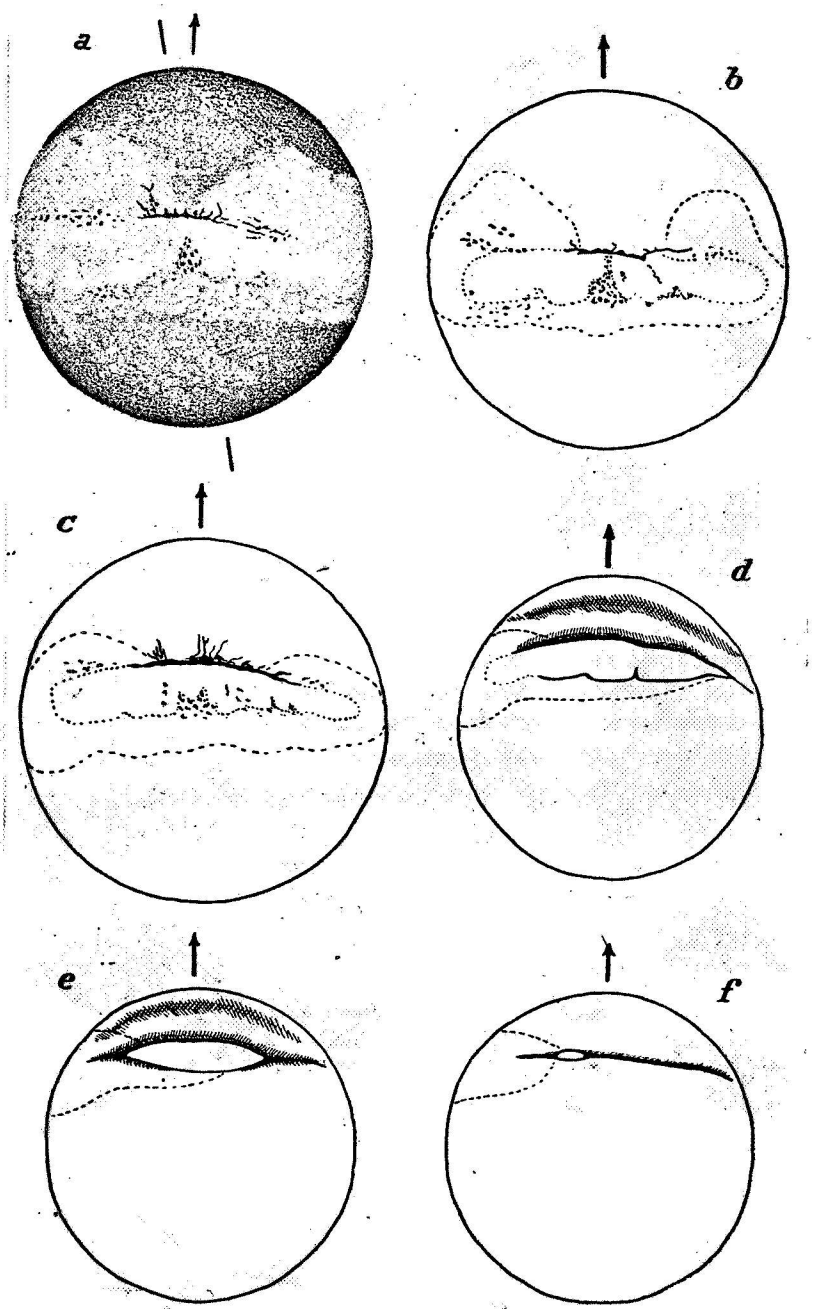

Figure 7. Embryo I 18. a, March 9 at 10:50 a.m.; b, at 12:00 noon; c, at 2:30 p.m.; d, at 7:05 p.m.; e, at 9:45 p.m.; f, March 10 at 12:45 a.m.

edge of very white material and are obviously induced by this material. /371
The dorsal anlage appeared first on all the embryos observed. Its location depends upon the dorsal region of the original gray crescent. The ventral anlage, on the other hand, is obviously not related to this region. Both anlagen form an enclosure around a strip of very white material and cause the formation of an initially wide groove, which later closes up to become narrow. It is possible that, when the very white mass is very wide, a roundish blastopore may be formed from two originally separated blastopore anlagen. The remaining conditions are similar to those already described for other embryos.

Chiarugi also described the formation of two blastopore anlagen which were originally located far apart, and their later amalgamation into a linear blastopore slit (see p. 11). This observation is valid, but our experiments showed that the occurrence of such a form of gastrulation in Rana is rare, even though we especially sought out such cases and treated them in detail. Dürken also assumes, in his schematic description of gastrulation, the existence of two gastrulation splits, which are initially located far apart from each other. He supports his assumption on the information furnished by Wetzel, who did not, however, describe such a phenomenon at all (see p. 13).

3. Formation of a Groove Running in a Straight Line, which Was Initially Narrow and which either Had no Yolk Plug or a Small one

An initially wide groove later becomes very narrow because its lips grow towards each other (Figure 7d-f). The characteristic indication of the blastopore form to be treated here is that which occurs from the beginning as a narrow groove. Otherwise, its method of formation varies greatly, as will be seen in a few examples.

a. Formation of the narrow groove after formation of a seemingly normal dorsal blastopore lip

This type of groove formation is very similar to that shown in Embryo X 11 (Figure 6). The blastula shown in Figure 8a (Embryo I 1) was developed from a rotated egg whose first furrow lay exactly frontally. A light band ran in an exactly median direction across its vegetal surface and was therefore perpendicular to the first furrow. A strip of very white material was located within this white band exactly in its longitudinal direction. Gastrulation began in this embryo in a manner not dissimilar to that of a normal embryo. Exactly at that spot, which was indicated by marking as the center of the original gray crescent, a small arcuate depression formed. It differed from the anlage of a normal /372
dorsal blastopore lip in that it was shaped more in the form of a horseshoe and lay somewhat closer to the equator of the egg. The location of

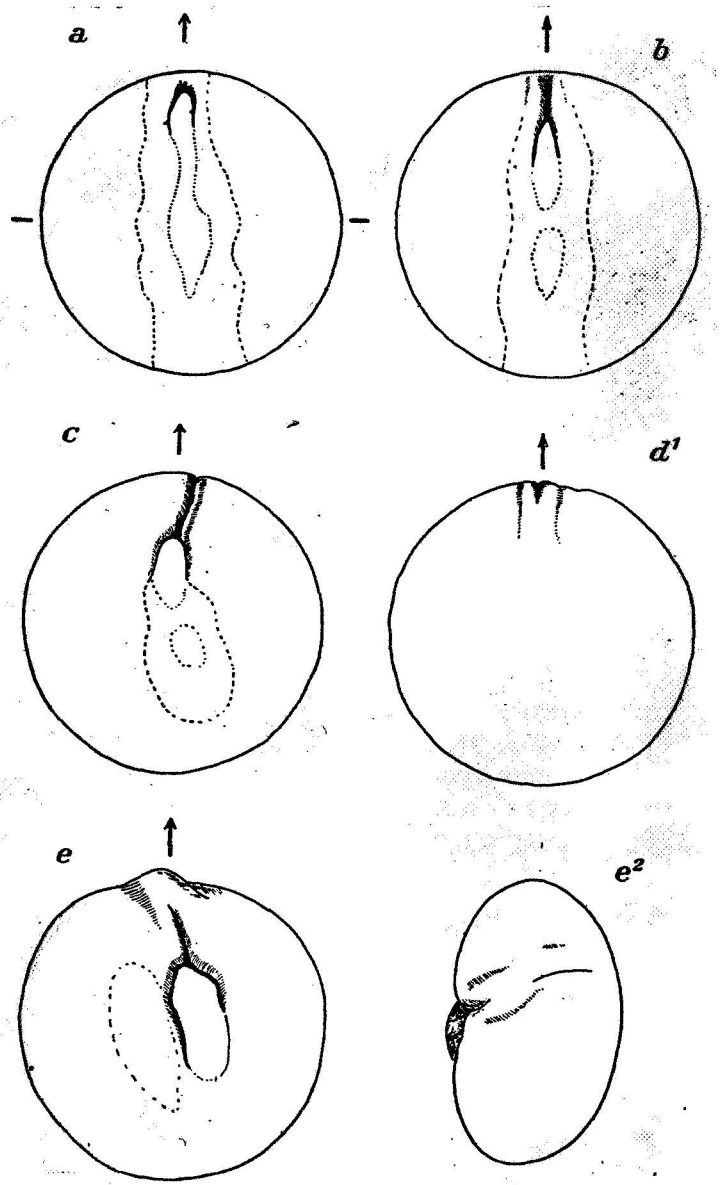

Figure 8. Embryo I 1. a, March 9 at 10:10 a.m.; b, at 2:20 p.m.; c, at 6:45 p.m.; d^1, at 7:40 p.m.; e and e^2, March 10 at 12:45 a.m.; e^2 is a view of the dorsal edge

its formation was also unmistakably related to the mentioned strip of very white material in that the arcuate depression surrounded the dorsal end of this strip. Instead of first forming a semicircle and then a completely closed circle, this blastopore anlage formed a groove. The /373

shanks of the horseshoe-shaped depression extended and applied themselves against each other at the bending point in a manner like the right and left blastopore lips normally should, according to the well-known concrescence theory. For this reason, a narrow groove was formed immediately, whose lips diverged in a ventral direction and still surrounded the dorsal end of the white material as before as far as it was visible (Figure 8b).

A comparison of the two figures definitely shows that this process causes the dorsal portion of the pure white material to shift into the interior of the embryo in that it is surrounded like a yolk plug by the lips of the blastopore anlage and is submerged between them. One observes further that also the other portions of the white band (namely, at first the portion to the left of the groove) which has formed becomes narrower. This may only be explained by its tucking-in around the left edge of the groove. The lengthening of the blastopore anlage in a ventral direction continued in the same manner, causing the narrow groove developed by the close application of the lips to become even longer. An ever greater portion of white material between the diverging shanks of the blastopore was submerged into the interior of the embryo (Figure 8c). The narrow groove also lengthened in a dorsal direction, so that it now cut into the dorsal blastula edge and extended a short distance onto the animal surface of the embryo (Figure 8d[1]). The white material on both sides of the narrow groove on the vegetal side had partially disappeared, meaning that it had obviously tucked in around both lips of the groove and penetrated into the interior of the embryo. The two shanks of the blastopore anlage completely surrounded the remnant of the very white material, so that this portion of the anlage resembled a normal round blastopore and surrounded a yolk plug (Figure 8e).

The closing of this portion followed in the manner described previously. The lips came together from the dorsal to the ventral side over the yolk plug, whereby the plug continued to submerge and therefore became smaller and smaller (Figure 9f). This occurred during a time when the entire blastopore anlage was extending toward the ventral edge of the embryo. When the entire yolk plug had been submerged and the blastopore lips had closed into a narrow slit above it, the whole structure was located very close to the ventral edge (Figure 9g). A residue of the white band, which had not been tucked in around the lips of the groove, was still visible. The closing of the groove had already begun at this time. The groove was no longer recognizable on the dorsal edge for a time (Figure 8 and e[2]), but later reappeared at this point (Figure 9f). The portion of the groove on the animal surface disappeared first, obviously through the growing together of the lips. After this, the groove closed up also on the vegetal side in a direction from the ventral to the dorsal (Figure 9h and i). Only a small slit remained from the entire blastopore anlage. It was located in the place where

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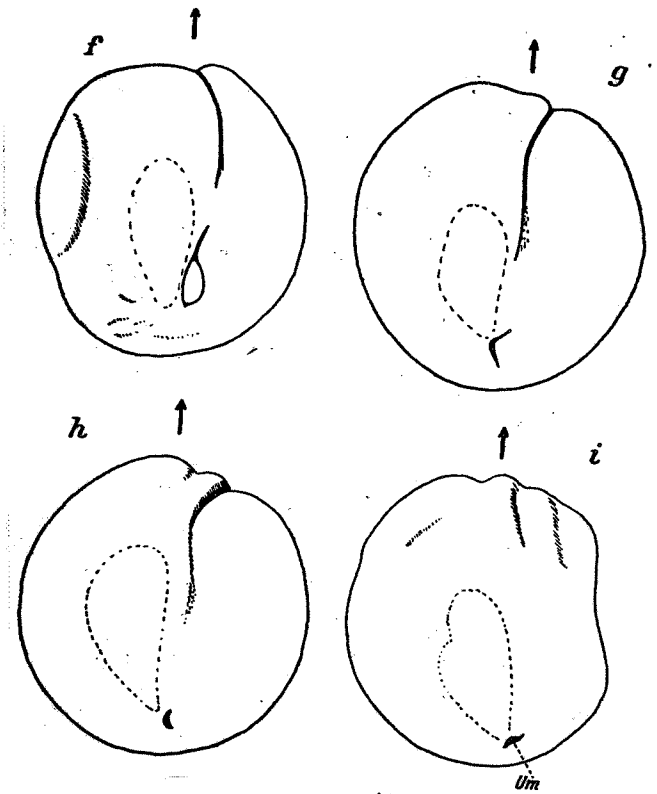

Figure 9. Continuation of Figure 8. f, March 10 at 5:45 a.m.; g, at 11:00 a.m.; h, at 2:30 p.m.; i, at 7:30 p.m. Um, remainder of the blastopore

the lips surrounding the yolk plug had last united over it (Um in Figure 9g-i).

We will give only one more photograph of this type of groove formation, which was made from another embryo (17 (25)). This embryo, with the first groove in a frontal position, also had a white band running in the direction of the median. This band was wider on the dorsal side and protruded somewhat onto the animal side (Plate VI, Figure 7a). /375

The first blastopore anlage and its further development was sketched and, just as the embryo reached the approximate stage (Figure 8b) of Embryo I 1, it was photographed (Plate VI, Figure 7b). One sees how the narrow horseshoe - shaped blastopore anlage encloses the dorsal end of the very white material with its two shanks (as long as the white material is still visible on the surface at this stage), and then continues toward the dorsal edge in the form of a narrow slit. This last occurrence may only be seen through a magnifying glass. A comparison of the

two photographs makes it clear just how much of the white material has already reached the interior of the embryo. Part of this material reaches the interior by tucking around the lips of the blastopore anlage and the rest by submersion between them.

Summary. The embryo in Figure 6, which is described above, differs from that shown in Figures 8 and 9 (and also the embryo shown in Plate VI, Figure 7) in the following manner. The very white strip in Figures 8 and 9 was very narrow. The blastopore anlage, which occurred on the dorsal end of the strip, was therefore very short and had a horseshoe shape initially. The shanks of this horseshoe-shaped groove also surrounded the very white strip on both sides, but they were located much closer to each other as a result of the narrowness of the strip. They also moved closer to one another as they extended their lengths. This caused the initial formation of a very narrow groove instead of one which was wide at first.

b. Formation of the blastopore starting as a narrow groove

The immediate appearance of a narrow, straight groove is the most conspicuous form of gastrulation in rotated embryos because it is the most typical type occurring in such embryos and because it deviates from the norm to the greatest degree. We followed the course of this form of gastrulation in manifold variations in a large number of embryos, and present a few characteristic examples.

Embryo IV 11 had a frontal first furrow and, in the blastula stage, a white band running almost exactly median. This band extended over the entire vegetal surface, and on its ventral edge it reached rather far over onto the animal surface (Figure 10a and a^1). In the middle of the white band, there is a dark brown, pigmented line which runs in the band's longitudinal direction. This line was continuous in the dorsal portion, but was still intermittent in the ventral portion. Our experience with other embryos allowed us to make the probable conclusion that /376
the first blastopore formation, which was not observed in this embryo, occurred dorsally in the white band, and therefore in the middle region of the original gray crescent. This pigment line deepened into a very thin slit and thereby curved slightly around the dorsal edge of the em- /377
bryo (Figure 10b and b^1).

The development of the groove in the direction of the ventral edge, where very white material was visible in a white band, was somewhat retarded. This white band was surrounded like a yolk plug by the groove's lips, which were separating in a ventral direction. This was also clearly visible on the animal side of the embryo (Figure 10c and c^1). In a somewhat later stage of development, the plug became clearly smaller on the animal side, because the blastopore lips had begun to grow together

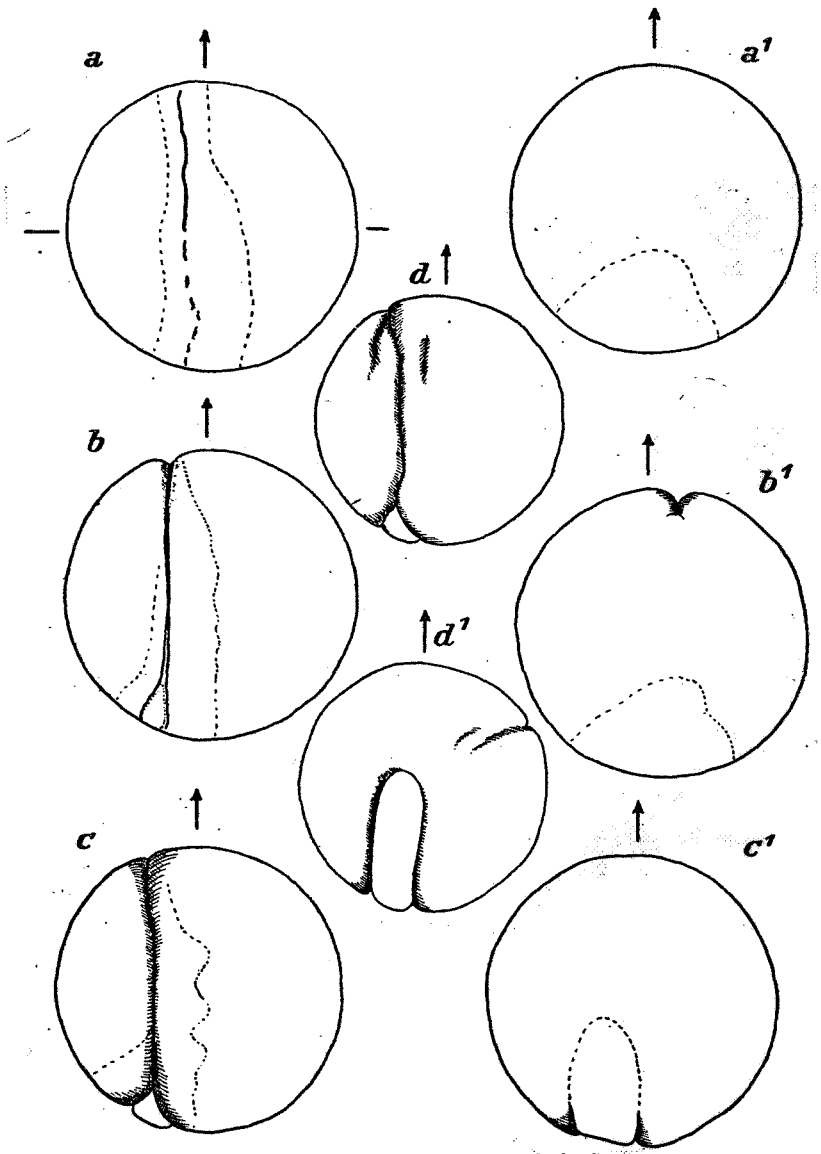

Figure 10. Embryo IV 11. a and a^1, March 16 at 2:20 p.m.; b and b^1, at 7:30 p.m.; c and c^1, March 17 at 1:00 a.m.; d and d^1, at 6:25 a.m.

over it (Figure $10d^1$). The tucking-in of the other portions of the white band is also clearly visible here. That portion of the white band to the left of the groove has completely disappeared, except for a small part on the ventral edge. This may already be observed in Figure 10b and is even more pronounced in Figure 10c. In Figure 10d, nothing more may be seen of the white band with the exception of the yolk plug. Its disappearance

may only be based on the fact that it tucks in around the right and left edges of the deepening groove and moves to the interior of the embryo. The closure of this groove was not followed on this embryo.

On Embryo IV 29, the first furrow was oblique to the median plane. A white band developed perpendicular to this furrow. This band was also, therefore, oblique to the median plane (Figure 11a). It continued around the ventral edge, becoming wide, and extended over onto the animal surface (Figure 11a[1]). The blastopore anlage appeared in the approximate middle of the band in the same direction. In the dorsal portion the blastopore anlage appeared as a narrow slit which curved around the edge. In the ventral portion it first appeared as a continuous line, which also could be recognized as a groove on the ventral edge in that it formed a notch there. This notch then developed into a continuous narrow groove which ran across the entire vegetal surface, continued into the animal surface, and surrrounded the larger portion of the end of the band which was composed of pure white material (Figure 11b and b[1]). The white band had clearly become narrowed, which is explained by the fact that its material had tucked in around the edges of the groove into the interior.

Finally, the pure white complex was completely surrounded and the groove lengthened until it surrounded the embryo in the shape of a ring (Figure 11c and c[1]). The pure white material was then located on the animal surface in the form of a long, yolk plug on its ventral edge between the two lips of the groove. Subsequent to this, these lips opened somewhat so that some white material was visible here also in the base of the groove. Eight hours later, the groove on the vegetal surface /378
gaped widely, showing a whitish base, whereas it remained narrow on the animal surface at first and surrounded only the yolk plug which had become smaller in the meantime (Figure 12d and d[1]). Finally, the groove /379
also widened on the animal side so that it surrounded the entire embryo in the form of a wide furrow with a whitish-gray base (Figure 12e and e[1]). The embryonic anlage appeared in this stage of development.

The embryos shown in Figures 10b and 11b are very similar to those in Figures 35 and 36. They also have a narrow groove which forks at one end and surrounds white material as a yolk plug in that area. In the embryo in Figure 34, the groove forks on both ends and thus surrounds two yolk plugs. This would certainly also have occurred in the embryo shown in Figure 18 since a light white complex also lies at each end of the narrow groove, and the lips of the groove would have subsequently enclosed both complexes, just as we actually observed in many other instances.

The formation of the narrow groove on Embryo XI 2 (Figure 13) was not so typical. The first furrow was located in a median position, and a white band perpendicular to it, and therefore also frontal to it, was formed (Figure 13a). This white band continued onto the animal surface

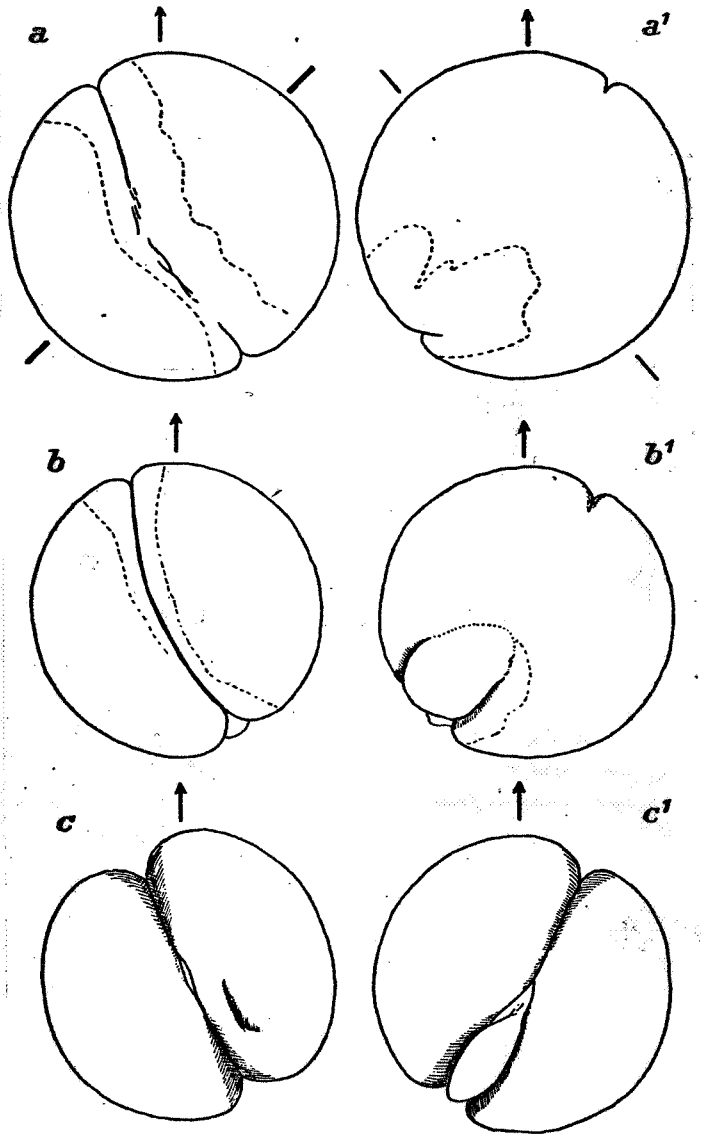

Figure 11. Embryo IV 29. a and a^1, March 16 at 8:30 p.m.; b and b^1, March 17 at 2:00 a.m.; c and c^1, at 7:00 a.m.

around the left and right edges (Figure 13b^1). Within the two ends of the band a very white area similar to that seen in Figure 18 could be recognized. The groove then disposed itself into two distinct sections, a right and a left. /380

The first two anlagen of the blastopore lips are located, as is shown in Figure 13a, in the two horns located in the right and left side

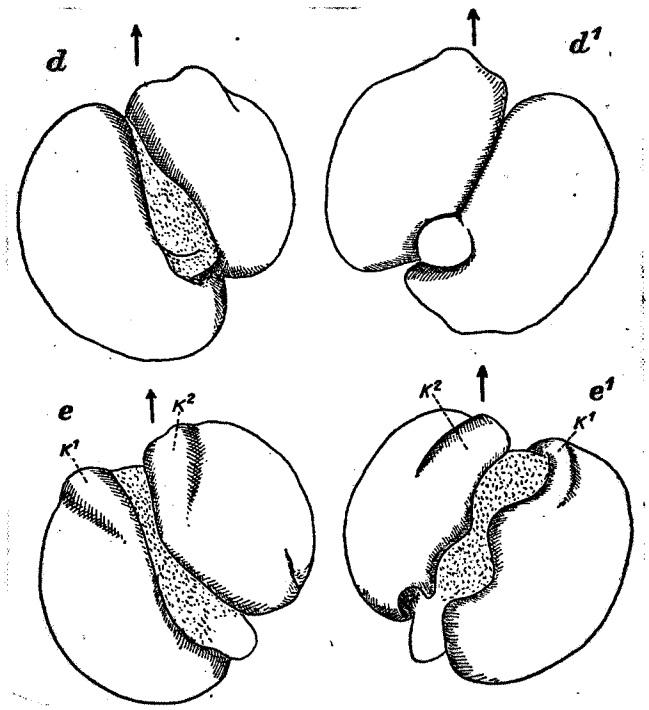

Figure 12. Continuation of Figure 11. d and d^1, March 17 at 5:00 p.m.; e and e^1, at 9:00 p.m.

portions of the original gray crescent. The right anlage was a dark pigment line running along one side of the very white area on the right. The left anlage was somewhat further developed at this time. It appeared as a slit stretching from the inner end of the very white area on the left toward the general direction of the middle of the vegetal surface; however, towards the left it opened into two lips which already surrounded a portion of the very white material. This left anlage, therefore, was exactly the same in appearance as the anlage shown in Figure 8a. It then surrounded the light white material in the left end of the band completely by reaching over onto the animal surface (Figure 13b and b^1). Its right, groove-like end then lengthened, finally joining the right blastopore anlage (Figure 13c). No yolk plug had been formed on the right. The white material located there had disappeared into the interior by some means and the blastopore anlage had immediately developed into a groove, which extended just around the right edge of the embryo (Figure $13b^1$). The white band on the middle of the vegetal surface had already disappeared at this stage, but was still visible on the animal side and on the right and left of the vegetal side. It narrowed on the right and left of the animal side in the direction of the center. One had the clear impression, that the material in the white band in the middle of the vegetal surface had tucked in over both groove

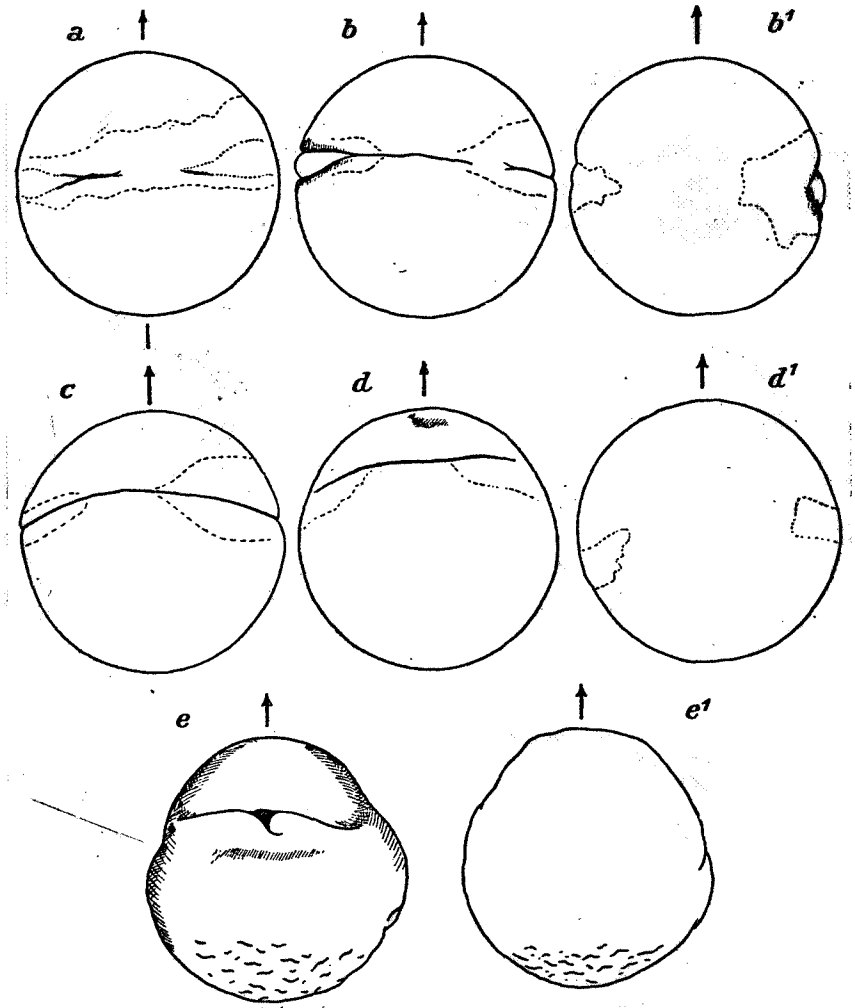

Figure 13. Embryo XI 2. a, April 1 at 3:30 p.m.; b and b^1, at 9:30 p.m.; c, April 2 at 1:45 a.m.; d and d^1, at 12:30 p.m.; e and e^1, at 7:00 p.m.

lips into the interior of the embryo, while such tucking had not occurred either on the animal side or on the right and left of the vegetal surface. At this point, the closing of the blastopore groove began. The yolk plug on the left was already completely submerged at the stage shown in Figure 13c, and now the groove shortened from both ends (Figure 13d). Further material from the band must have tucked into the interior before this shortening, since the size of the band on the animal surface decreased greatly and, on the vegetal surface, that portion dorsal to the groove had disappeared completely (Figure 13d and d^1).

A further shortening of the groove occurred during the stage shown on Figure 13e and a wider depression has replaced the slit in the middle. On the vegetal and animal ventral sides many small wrinkles have appeared (Figure 13e and e^1). These are unquestionably caused by an increase in the area of the surface of the embryo. Since they are not found on all embryos, and since, when they are present, they are located in the most various spots and are developed to different degrees, we would like to point out in this connection that the increase of the embryo's surface area does not always and in all places correspond to the tucking-over around the groove lips to a sufficient degree. A few additional photographs of gastrulation stages should afford a true concept of this type of blastopore formation. /381

Embryo 13 (25) is shown in the blastula stage and at the beginning of gastrulation (Plate VI, Figure 4a and b). The first furrow also had a median position and the white band likewise had a median course. A slit appeared somewhat to the left of the middle line of the white band, in the middle region of the original gray crescent. A comparison between the a and b parts of Figure 4 shows that the white band had narrowed considerably in this area, obviously caused by the tucking of material around the left edge of the groove. /382

In Plate VI (Figures 9b and 10a) as well as in Plate VII (Figure 11a) the very beginning of the formation of the groove is to be seen. It appears as a fine, dark brown pigmented and irregularly branched line, which seems already to be somewhat deepened in Figure 9b. This may be seen clearly when the photographs are viewed with a magnifying glass. Figures 10b and c (Plate VI) shows the further development of such a groove. On this embryo, it was formed on the right boundary of the lighter strip in the white band, and it deepened and lengthened at its two ends. One may also recognize further in these pictures that the white material decreases greatly in dimensions even at the onset of gastrulation. Above all, a comparison of parts a and c of Figure 10 shows how the white band decreased greatly in size in an area to the right of the groove. This decrease began at the start of gastrulation and was obviously due to the tucking of surface material around the right edge of the groove. The very white material had also substantially decreased to the left of the groove. This may easily be seen with respect to the very white material located to the left of, and dorsal to, the groove. The white material in this case was not submerged as a yolk plug, but had obviously also tucked in around the edge of the groove.

The embryo in Figure 11a (Plate VII) shows, as we have already mentioned, the beginning of groove formation. In this case the band and the groove have frontal locations. One sees very clearly that the onset of the groove formation occurred on the dorsal boundary of the very white strip within the white band. Plate VII (Figure 11b) shows a later stage of the groove development. The pure white material previously seen (Figure 11a) appears to form a yolk plug on the right edge.

Figure 13a (Plate VII) shows a relatively rare picture of the vegetal surface of a blastula. This has already been mentioned on page 46. The first beginnings of the blastopore and the further course of gastrulation in this embryo were completely in accord with the determinations previously described by us. It began in the form of a short, oblique slit located exactly on the vegetal edge of the middle of the original gray crescent, which is the normal location of such a blastopore. At the points where this slit encountered the very white material of the vegetal surface (namely, at its ends on the right and left) it forked and surrounded both very white complexes, enclosing them like a yolk plug and then submerging them as such (Plate VII, Figure 13b). This picture corresponds, therefore, to the one in Figure 32. /383

Embryo 22 (25) also shows the formation of a groove which was narrow from the beginning, but in which a conspicuous yolk plug is submerged. It was photographed in a saucer without previous determination of the median direction. At first a somewhat longer pigmented and branched line appeared within a rather irregularly outlined light field on the vegetal surface (Plate VII, Figure 14a). This line was already deepened in a groove-like formation, and bordered on the right side of the dorsal edge of a very white complex. This complex was enclosed by this line and one of its branches in the form of a yolk plug. Plate VII, Figure 14b shows this typical groove in which the white yolk plug has just been submerged. The groove has reached its longest extension in this stage and, after the plug had been submerged, it began to close, starting from its two ends (Plate VII, Figure 14c). Finally, only a short slit remained in the middle of the vegetal surface (Figure 14d). The earlier groove corresponded in location and dimensions to a bulge formed by a sinking-in process on both sides of the groove and which bore the slit in its center.

Figure 15 (Plate VII) shows, in conclusion, the appearance of a long groove. It extends across the entire vegetal surface oblique to the median plane, and ventrally on the right somewhat onto the animal surface in the form of a thin slit. It ends dorsally to the left in the form of a fork at a large complex of very white material (Figure 15a and a^1). The very white material was completely surrounded during the further course of gastrulation. This was accomplished through an extension of the edges of the fork-shaped formation onto the animal surface (Figure $15b^1$).

Summary. If no very white complex is located on the vegetal surface of the blastula, or if gastrulation begins at least in an area where no such complex is present, the blastopore is formed from the beginning as a narrow groove running in a straight line. It first appears as a more or less branched, darkly pigmented line, which soon deepens into a very fine slit. Instead of one such line and one such slit, two or three may occur. In some embryos, which will be discussed later, an even

larger number of such lines and slits were observed. In any case, they /384
are all oriented in the same direction and soon join to form one line and one slit. The areas of predilection for the first appearance of this blastopore anlage are the region of the original gray crescent (its middle region or its horns) and the border areas of smaller zones of very white material. Exceptions are discussed later in this study. Lighter material tucks in around both lips of the deepening groove and penetrates into the interior of the embryo. This tucking does not occur uniformly along the entire length of the groove. In places where the groove meets a complex of very white material, it forks around it and submerges it between its lips in the manner of a yolk plug. For this reason, one very often finds such a yolk plug at one or both ends of the groove. The groove may be short from the start or may be long, in which case its entire length may appear simultaneously or it may be formed from the joining of many separate sections. It may maintain its approximate original length or it may extent in a direction more or less coincidental to its original direction. As a result of this, it may be confined to the vegetal surface or it may reach around one or both edges onto the animal surface. In extreme cases, it may encircle the entire embryo in the form of a "circular furrow" (Schultze and Wetzel).

In the cases already discussed here, the narrow groove appeared in the longitudinal direction of a more or less clear white band. Embryos to be discussed later also show the occurrence of the groove without the presence of a white band; however, as far as we can ascertain, they always appear in relationship to light white areas which are more or less distinct and arranged in the form of strips. See the next section for information concerning the orientation of the groove to the median plane and to the first furrows. The fate of this narrow groove is the same as that of the groove which was wide at first. It closes either from one end to the other, or from both ends toward the middle. The narrow groove which does not close at all, may subsequently become wider. The next section will also discuss the formation of wrinkles.

4. Formation of an Animal Blastopore Anlage Independent of the Vegetal Anlage

In rare cases we observed the appearance of a slit on the animal surface, which was completely independent of the blastopore appearing on the vegetal surface. This slit was completely similar to a blastopore groove.

Embryo VI 5 did not have a white band in the blastula stage, but /385
rather a somewhat roundish, lighter field (Figure 14a) containing an elongated, pure white zone in the median line in its ventral region. It must be mentioned that, because of the complexity of the surface

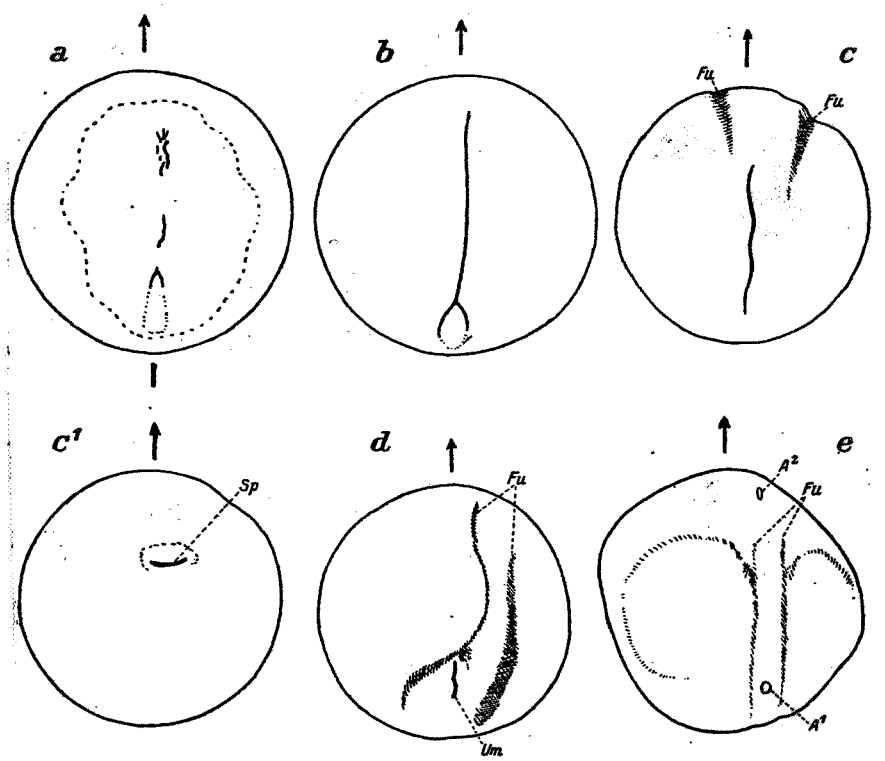

Figure 14. Embryo VI 5. a, March 23 at 9:30 p.m.; b, March 24 at 8:00 a.m.; c and c^1, at 2:10 p.m.; d, at 8:30 p.m.; e, March 25 at 12:15 p.m.

Fu, furrows; Um and A^1, remnant of the blastopore formed from the groove; A^2, remnant of the blastopore formed from the animal blastopore slit (Sp); A^1 and A^2 lie on the opposite side of the picture shown in part e of this figure

aspect, our sketches show only the more pronounced pure white areas. It is entirely possible that, in reality, other smaller pure white areas were present.

The picture shows the first blastopore anlage which appeared in three places in the median line in the form of thin pigment lines. One was able to observe that the very white areas would be surrounded by fork-like extension of the line. During the process of further development, the pigment lines extended and deepened into a continuous groove which stretched across almost the entire vegetal surface in a median direction. It surrounded the white place on its ventral end (Figure 14b),

which then submerged as a yolk plug and disappeared (Figure 14c). Simultaneously, the groove shortened from its dorsal end and became a short slit on the ventral edge (Um in Figure 14d). This slit then reached around the edge onto the animal side and finally arrive at the point designated as A^1 in Figure 14e.

We were not able to determine just how this migration proceeded. It is not impossible that the short slit in Figure 14d lengthened constantly in the direction of the ventral edge and then extended around it to point A^1 on the animal surface, while, simultaneously, it continuously closed up to the same degree at its other end.

The embryo described shows two further pecularities. As we have often observed, deep and very conspicuous furrows appeared on its surface during gastrulation. At first, one furrow each was observed (Fu in Figure 14c) on each side of the groove, on the dorsal edge of the vegetal side. Both furrows extended in the direction of the ventral edge, deepening at the same time. All of this occurred to the same degree as the closure of the groove proceeded (Figure 14d). These furrows were later laterally bounded by two protuberances which approached the closing groove on both sides and which possibly belong to the embryo anlage (Figure 14e). Very similar grooves occur in other embryos, but they are probably, at least partially, subject to another interpretation.

The second peculiarity of this embryo is the one treated in this section of the study. During the time in which the described groove was confined to the vegetal surface, a short, narrow slit (Figure 14 c^1) /386 appeared in the middle of a small complex of whitish material on the animal surface. This slit at first remained unchanged for a long time. The whitish complex disappeared gradually (through tucking) and the slit moved toward the dorsal edge to point A^2 on the animal surface (Figure 14e).

Summary. A small slit may appear on the animal surface, which is completely independent of the blastopore anlage on the vegetal surface. The location of this slit has no relationship to the region of the original gray crescent, but forms in the described place and in others in a light area which was visible on the animal surface. Since the light material in its vicinity later disappeared, it may be assumed that it penetrated into the interior of the embryo by tucking-in around its edges. If this is true, the slit must be designated as an animal blastopore anlage. There are cases (Figure 15) where the animal slit is not /387 surrounded by lighter material. It is possible that the slit was discovered at too late a stage in such instances; i.e., at a time when the lighter material was already tucked in. In Part V we shall discuss the

question of whether or not the animal slit sometimes does have some type of relationship with the vegetal blastopore groove.

5. Atypical Gastrulation Forms

The seemingly normal, roundish blastopores which are initially wide or which appear from the beginning as a narrow groove, may be designated as the typical gastrulation forms in the rotated embryo. Even the animal blastopore anlage is a characteristic, but rare, form of gastrulation. Among the large number of embryos in which we followed the gastrulation process, a high percentage either deviated from these typical forms so greatly that they were unclassifiable, or they combined two of these typical formations, or they changed from one type to another during gastrulation. Part V presents many examples of these. In this section we shall therefore confine ourselves to the description of two such phenomena.

In Embryo I 19 (Figure 15), a narrow blastopore groove was initially formed which then evolved into a seemingly normal, roundish blastopore. With the first furrow in a median location, it formed a narrow frontal groove in a frontal white band. The ends of this groove each forked around a yolk plug (Figure 15a). The lighter material of the band disappeared over the edges of the groove, while the yolk plug remained visible (Figure 15b). The groove subsequently became wider along a major part of its course, so that the white yolk material between its lips was exposed, whereby further tucking over the ventral edge of the groove obviously occurred at the place marked with an arrow in Figure 15c.

This last tucking process miscarried somewhat: the brown, spotted material, which should have been tucked in over the ventral edge of the groove, was afterwards located in the base of the groove between the two yolk plugs (Figure 15d), and a new enveloping edge was formed ventral to it. The yolk plugs and the brown-spotted material were then surrounded by an approximately oval blastopore lip (Figure 15e) because of the simultaneous widening and shortening of the groove. This blastopore lip became even rounder as it closed over the submerged material (Figure 15f). It did not, however, close completely; the right end of the original groove could still be recognized in the stages shown in Figure 15, d and e. It was flanked by two furrows, which are evidence of a swelling along this end of the groove (Fu in Figure 15d) which is obviously closing from the right. This swelling could be seen much more clearly later along the completely closed end of the groove (Figure 15f). /388 /389

If one imagines that this right end of the groove has extended around the right edge of the embryo onto the animal surface, then a

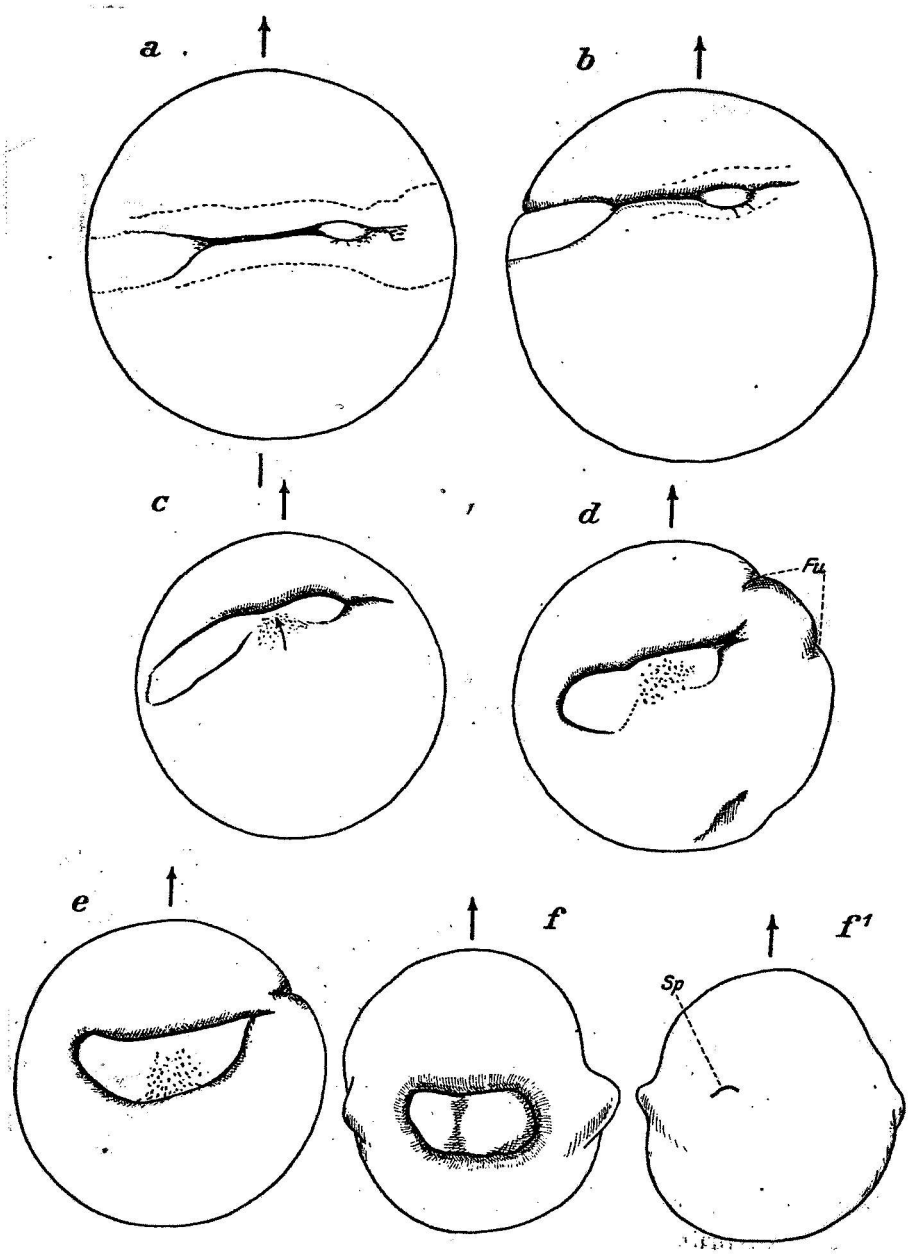

Figure 15. Embryo I 19. a, March 9 at 2:20 p.m.; b, at 4:45 p.m.; c, at 7:05 p.m.; d, March 10 at 2:45 a.m.; e, at 6:00 a.m.; f and f^{1}, March 11 at 9:00 a.m.

Fu, furrow; Sp, animal blastopore anlage

point is reached where a seemingly independent slit has been formed (Sp in Figure 15f[1]). This did not lie within a lighter area as in Figure 14c[1]. We have mentioned above that, at the relatively late time that this slit was recognized in the course of checking, light material might possibly have penetrated into the interior of the embryo. We may therefore state that the formation, which appears in Figure 15f to be a relatively normal blastopore, is nothing more than a portion of the original narrow groove, which greatly extended itself in subsequent stages of development.

When one observes the stage of development of Embryo IV 13, shown in Figure 16f[1], one would prefer to consider the blastopore as relatively normal, with the exception of the peculiar yolk plug. In reality, this blastopore was formed in a peculiar and extremely atypical development process. The embryo had a first furrow running in a median direction and a frontal white band in the blastula stage, similar to that shown in Plate VII, Figure 13a; this band was almost broken in the middle (Figure 16a), but extended right and left onto the animal surface (Figure 16c[1]). The first beginning of the blastopore is seen as a short, frontal pigment line in Figure 16a. From this line a rather long frontal groove developed, which submerged two light places as yolk plugs between its lips (Figure 16b). It could be clearly determined in this picture that a portion of the light material had already tucked in around the lips at this stage. As could be expected from the results obtained on all the other embryos previously studied, the groove extended in the direction of the white band and thereby reached around over the right and left edges onto the animal surface (Figure 16c and c[1]).

There was, in this case, one other peculiarity which bears mentioning. The first blastopore anlage corrsponds roughly to the vegetal boundary of the dorsal region of the original gray crescent (Figure 16a). The further developed groove shifted to a position much closer to the dorsal edge (Figure 16, b and c). It moved even further in this direction in the stage shown in Figure 16, d and d[1]. It slid to one side like a rubber band placed around a sphere, simultaneously contracting, and developed into a complete ring during this process. This "sliding" occurred in such a manner that the entire course of the groove came to rest on the animal side of the embryo. For this reason, it may be seen completely on this side in Figure 16e[1]. At this point, it had not closed completely into a ring. Its right end has formed a fork around a small yolk plug. Its complete closure into a ring is prepared by the /390
independent formation of a small groove between its ends. Complete closure of the ring was prepared, in that between its two ends a small groove segment developed independently and was completed in the stage shown in Figure 16f[1]. The ring became smaller and smaller until it developed into a rather normal anus.

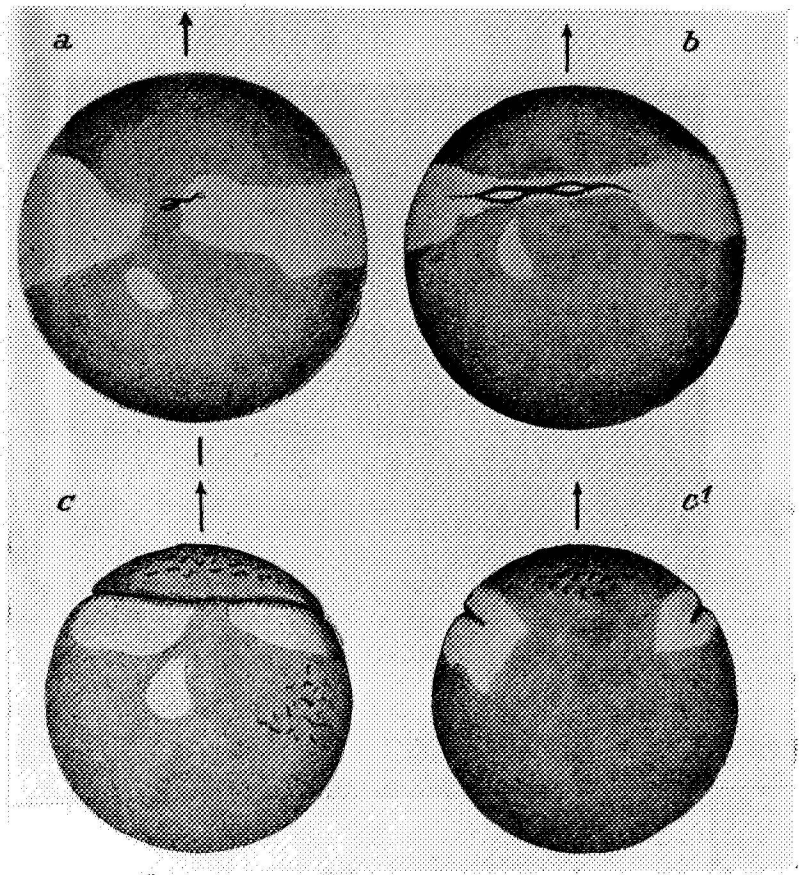

Figure 16, a-c[1]. Embryo IV 13. a, March 16 at 2:30 p.m.; b, at 7:30 p.m.; c anc c[1], March 17 at 12:30 a.m.

One may observe in the figures the extent to which the material in the light band has tucked in around the lips into the interior during this process. The formation of wrinkles was very conspicuous on this embryo. It occurred at first on the right side of the embryo, which indicated to us that the tucking-in of material around the ventral edge of the groove did not keep pace with the surface area increase of the embryo in this region. The formation of wrinkles was very extensive in the dorsal region (Figure 16, c and c[1]). This wrinkled area was raised into a warty, conical protuberance during the period in which the groove was developing into a ring, which was growing ever smaller /391
(Figure 16, e and e[1]). This was unquestionably caused by the fact that the entire region dorsal to the groove was pushed together in spite of its inherent superficial growth. Simultaneously, this formation was submerged as a yolk plug within the ring-like groove, or, more exactly, within the ventral lip of this groove (Figure 16c[1]). One may see both

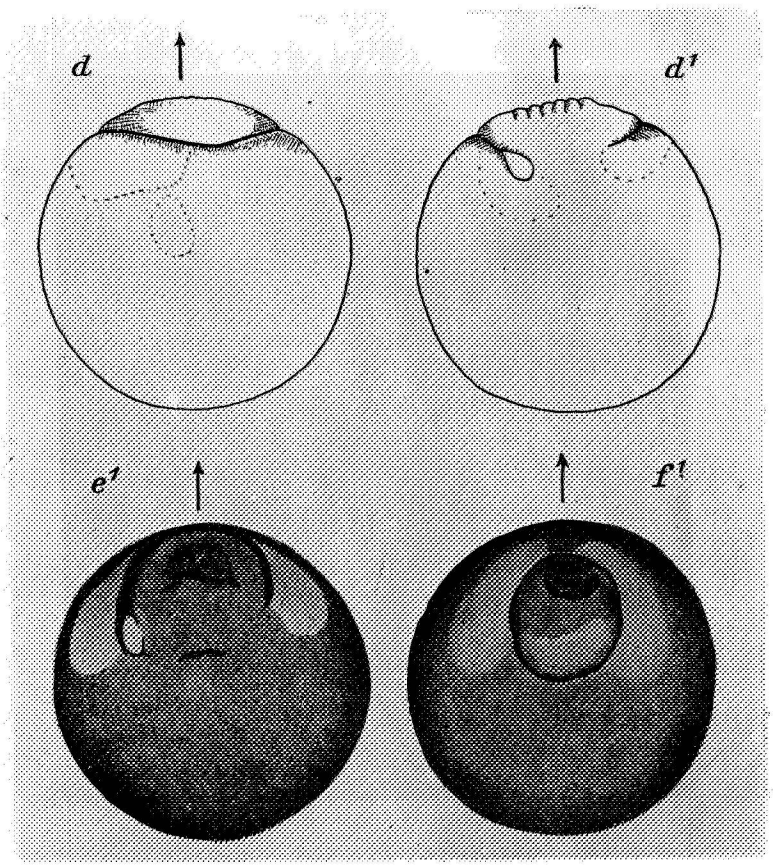

Figure 16, d-f[1]. d and d[1], at 5:30 a.m.; e[1], at 10:30 a.m.; f[1], 3:30 p.m.

the warty cone and portions of the lighter material of the original white band within the "yolk plug."

The round blastopore shown in Figure 16f[1] is therefore abnormal not only because of its dorsal location on the animal surface, but also because it is nothing more than the ventral edge of a typical groove. The ventral half of the embryo had swallowed the dorsal half as a yolk plug.

These two cases should suffice as examples of atypical gastrulation processes in rotated embryos. They serve to illustrate just how manifold the changes in typical gastrulation may be. /392

6. The Formation of Wrinkles and Furrows During Gastrulation

We have already mentioned the formation of wrinkles during gastrulation. It is based on the fact that the areas in question on the surface

of the embryo become covered with small folds and protuberances. These wrinkles are always confined to only one or two areas, but may be located in widely varying places according to the following: ventral in Figure 9f and much more pronounced in figure 13, e and e^1; to the right in Figure 6c; to the right and dorsally in Figure 16c; only dorsal in Plate VII, Figure 17d, and more clearly visible in Figure 74. Other examples of wrinkle formations may be found in various other figures in Part V. It would be difficult to interpret these wrinkles in any other manner than the one indicated above: the surface of the embryo is extended and the surface becomes wrinkled where this extension process is not compensated for by tucking (of the material into the interior over the lips of the groove or blastopore).

Since these wrinkles may be located in the most widely varied regions, it is certain that no particular area exists on the normal egg, which is especially prone to develop folds. It is probable, however, that the material shifting in the rotated egg changes one or another spot on the surface in such a manner that it becomes an area with an especially large extension of dimensions. One can observe that these wrinkles disappear again (compare, for example, Figure 6c with 6d and Figure 16c (right) with 16d). It appears that the inhibition of extension that is a condition for wrinkle formation ceases after a time. Thus, the hypothesis is reached that the tucking-in of material around the groove lips (a precondition for which is that an increase of embryonic surface area must take place) is in many cases and in many places insufficient to compensate for this increase in area, but that after an interval under certain conditions it could be resumed more actively. A very thorough study of such embryos would be necessary before the validity of this assumption could be proven.

The formation of furrows, which must not be mistaken for the gastrulation groove usually present, has also been mentioned in this study. Such furrows were observed in Figures 5e, 6d, 8, 9, and 10d, and were particularly pronounced in Figure 14d. They are found in widely varying areas, but especially nearly parallel to the gastrulation groove, in which case they border on the swollen lips of the groove. Photographs made of two embryos furnish an exact concept of this type of furrow, and also indicate that they can make a correct evaluation of the processes occurring on the surface very difficult, as Wetzel noted. /393

It would have been impossible to recognize the true blastopore lip in the embryo shown in Plate VII, Figure 16, if one had not followed the development from the beginning (its blastopore lip is marked as Ri). All other grooves shown are furrows, which occurred at a later time.

Figure 17a (Plate VII) shows an embryo with a furrow of medium length extending almost across the entire vegetal surface. The lips lie very close together dorsally, while they enclose a narrow elongated

yolk plug ventrally. The first stages of gastrulation appeared approximately as in Figure 7b (Plate VI). After submergence of the yolk plug, the groove became narrow in its ventral portion (Figure 17b). In the meantime the groove in the dorsal portion had closed and disappeared. Simultaneously, a depression already present, located to the left of the dorsal portion of the groove, had become significantly deeper and longer, so that it deceptively resembled a blastopore slit, especially in the dorsal portion. This phenomenon became even more pronounced as the blastopore groove shortened at its dorsal end (Figure 17, c and d).

Without exact knowledge of the development of such an embryo, one would probably size up these last two stages as follows. Left of the median plane a groove extends from the dorsal to the ventral edge. It forks approximately in the middle of the vegetal field in order to enclose a light complex there in the manner of a yolk plug (Figure 17c) and to submerge it (Figure 17d). In reality, however, the relationships are as indicated above, as is demonstrated by the study of the entire gastrulation process; in the stage illustrated by Figure 17d the gastrulation groove is rather short.

From this point on, the formation of furrows on this embryo changed significantly. Figure 17d shows, when compared with the previous stage (Figure 17c) that the furrow has already disappeared on the dorsal end to the left of the area of the groove. On the other hand, a number of wrinkles (Ru) have appeared dorsally and to the right of the groove. These wrinkles have already been mentioned above. A single, long furrow, which ran in a frontal direction, emanated from the area of these wrinkles and bounded a small swelling (Figure 17e). Simultaneously, the left furrow in the dorsal portion had closed completely. Its ventral portion had reformed into an arcuate furrow, which also bounded a swelling. In the vicinity of the ventral edge to the right of this last swelling, only a small remnant of the gastrulation groove remained in this stage of gastrulation (Um in Figure 17e). /394

These furrows are extremely difficult to interpret because they are very often lacking, and when they are present, their formations are very varied. Furthermore, the significance of these different forms certainly varies. They appear sometimes to be based on the same phenomenon as the wrinkles; namely, the inhibition of surface area increase due to insufficient tucking. In other instances they represent the early visible borders of the head anlage and the medullary fold, as will be shown in Part V. As we shall discuss below, the examination of sections indicated that, in many instances, they seem to have still another quite different significance.

7. Summary

The three partial processes of gastrulation

In normal gastrulation, the authors differentiate between three types of material displacement: 1, the invagination of the white yolk; 2, the tucking of the marginal zones around the lips of the blastopore; and 3, the surface area increase of the animal field and of the marginal zone. These three partial processes may be observed unquestionably in the gastrulation of a rotated embryo.

1. The submergence of the very white areas, which are enclosed like a yolk plug by the lips of the blastopore and then submerged, corresponds to the inversion of the white yolk. When seen solely from the outside, this picture resembles the normal submergence of the vegetal field even more. The larger the very white area, the more it resembles the normal vegetal field. On the other hand, if these very white areas are not present or are entirely lacking, no process occurs in the gastrulation of the rotated egg, which could be compared to the invagination of the white yolk in the normal egg, at least according to the instances discussed prior to this.

If no very white areas are visible any longer on the blastula, it is obvious that the entire white yolk has sunken into the interior without leaving any visible remnant on the vegetal surface. Furthermore, this sinking of the white yolk during cleavage has anticipated its later submergence in the form of a yolk plug. For this reason, we conclude that this sinking and the later submergence of the white yolk in the manner of a yolk plug, together correspond to the normal invagination of the vegetal field.

2. The tucking of the marginal zone around the blastopore lip are paralleled by the same processes in rotated embryos. During the formation of the blastopore, more or less large portions of the light band /395
unquestionably disappear in a completely different manner from the pure white areas. They are not submerged in the manner of a yolk plug. Their tucking in over the lips of the blastopore must be concluded from the appearance of the surface, since one may observe that the portion of the light band abutting against the edge of the blastopore becomes narrower and finally disappears completely. The assumption that this is the same type of tucking which occurs normally around the lips of the blastopore, will be further supported in the following section. This tucking process encompasses not only the material of the white band, but also darkly pigmented matter can also reach the interior of the embryo in the same manner.

This tucking may be clearly recognized only in the case of the white material, partially in the decrease in its extent, and partially by the fact that its cells are arranged in strands directed towards the lip of the blastopore. As we mentioned at the beginning, dark and light spots within the white band serve the same purpose as intravital staining tags would in such an experiment. The location and exact disposition of the blastopore determines where, and in which direction, material is tucked in around it.

3. The surface of a rotated embryo shows the same surface area increase as the animal field and the marginal zones of a normal embryo. It is the condition which allows the lips of the blastopore to close over submerged white material, and which allows surface material to be tucked in around these blastopore lips. The extent and direction of this area increase may not be determined with vital stain tags, as was used on Triton (Vogt, 1925). This has been explained in Part I, Section C. Since, however, the form and location of the blastopore is so variable, we shall see that the amount and direction of tucking must also vary. This leads to the definite conclusion that the exact type of surface area increase in rotated embryos must also be widely and individually varied. Two types of visual indications of this area increase may be observed.

First, the darkly pigmented surface of the embryo extends itself during the tucking of the white band or of the white formations of other types. Second, the occurrence of wrinkles and furrows is also indicative of surface increase. As we have mentioned, it may be assumed that these wrinkles and furrows are partially based on the fact that the surface increase is greater in scope than the tucking of material around the lips of the blastopore, causing the surface to form smaller (wrinkles) or larger (furrows) folds. The occurrence of wrinkles and furrows on the most widely varying areas of the embryo is an indication that the location and direction of the major surface increase varies widely on rotated embryos. /396

Location of the first blastopore anlage

In Triton, a small invagination pit in the vegetal field is normally the first blastopore anlage, located in the dorsal median line close under the vegetal edge of the lip zone: the dorsal blastopore lip which then develops corresponds to the edge described by Vogt (1922, 1926). In Rana fusca the latter has the same relationship: the dorsal blastopore lip normally indicates the vegetal edge of the gray crescent in the dorsal midline.

Following an investigation of the stages of rearrangement as well as of the blastulae, we have been unable to decide whether or not the

material of the gray crescent remains in situ in spite of displacement of the yolk material. If the first (sic) is correct, and if the gray crescent in the rotated embryo retains its special capabilities of form building and manifests itself, then it is to be expected that gastrulation will set in in the normal location in the rotated embryo also; i.e., since this location is no longer exactly to be determined after rearrangement, generally in a place that is determined through the original location of the gray crescent. In case it now results that gastrulation of the rotated embryo invariably or almost always begins at a specific place, the reverse conclusion follows; i.e., that the region of the gray crescent, although it is no longer identifiable, has not been disturbed by the rearrangement. Our observations actually led to this conclusion, but they also showed that the localization of the first blastopore anlage is determined by another factor.

The location of the first blastopore anlage was definitely determined on 32 of the embryos discussed in Part IV of this study. Of these, 25 formed only one blastopore anlage, six embryos formed two seemingly separate blastopore anlagen (Figures 7a, 13a, 20, 21, 22 and 30), and one embryo (Figure 14) formed four separate anlagen. Of the 25 blastopore anlagen which occurred singly in the embryos: (1) 17 lay exactly dorsal or slightly to the right or left in the region of the original gray crescent and simultaneously on the edge of a very white area; (2) three further anlagen had the same location, but not demonstrably on the edge of the very white area; and (3) five others did not lie in the region of the original gray crescent, but on the edge of very white material (four on the dorsal, and one on a lateral edge of this white material. /397

Of the six double blastopore anlagen, the following locations were noted: (1) in one embryo, one anlage in the right horn and the other in the left horn of the original gray crescent, both simultaneously on the edges of very white areas; (2) in three embryos, one anlage was located dorsally in the area of the gray crescent and simultaneously on the dorsal edge of a white mass, while the other was located on the ventral edge of this mass; (3) in two embryos, neither anlage was located in the region of the original gray crescent, but rather one was located on the dorsal and the other on the ventral edge of very white material.

Of the 37 individual anlagen mentioned thus far, not a single one was formed completely independent of either the region of the gray crescent or of the very white material. Twenty-three anlagen were formed dorsally in the gray crescent. All but three of these were simultaneously also related to the white material. Two anlagen were also located in the region of the gray crescent, but in its two horns, so that more than 2/3 of all these anlagen were related to the gray crescent,

and most were also related to the white material. Of the remaining 12 individual anlagen, all were formed, as we have noted, in relationship to white areas. Six of these, like a dorsal blastopore lip, were located on the dorsal side of the white area. Five others, like a ventral blastopore lip, were located on the ventral side of the white area, and only one was located laterally thereto. This revealing picture is not essentially disturbed by the conditions observed with respect to the embryo in Figure 14a. The four blastopore anlagen were located as follows: one dorsal in the gray crescent; one ventrally from the first, not located in the gray crescent and not definitely in the white material; a third anlage was located on the entirely ventral, but in the white material. These three anlagen are sections of the groove, which later becomes unitary. The fourth blastopore anlage was on the animal side and again related to a white area. The second animal blastopore anlage, described in Part IV (Figure 15f[1]), was found in a purely dark area. From experiences with other animal blastopore anlagen, it may be assumed that light material was also located here originally, but was overlooked.

The following results may be deduced from these observations. About 2/3 of all the individual blastopore anlagen observed were formed in the area of the original gray crescent and, almost without exception, were simultaneously in contact with the edge of a very white area. All other blastopore anlagen, which do not appear in the region of the gray crescent, are formed almost without exception on the edge of a very white area, usually being located on the dorsal or ventral edges. When we consider our experiences with other embryos not discussed here, we must conclude that even those blastopore anlagen forming on the animal surface are almost always also related to a lighter area, not observed here as a rule. /398

The embryos used in the compilation of the above summary were so chosen that one might question the validity of the statistical evaluation. The choice of embryos was made, as one may easily ascertain, so as to include, if possible, all the various types of blastopore anlagen. The typical behavior, the formation of such a blastopore anlage dorsal in the region of the original gray crescent, was somewhat neglected. This would also have been true, however, even if we had used all the embryos examined in compiling such statistics.

During the process of choosing those embryos to be controlled from the thousands of rotated eggs available to us, we found that the first blastopore anlage was almost uniformly located dorsal in the gray crescent. For this reason, we attached special importance to more detailed observations of deviating cases. Furthermore, two sources of error are to be taken into consideration in the determination of the location of the first blastopore anlage. First, as was mentioned in the introduction, it is very possible to be misled in this determination of the location

of the gray crescent. If the blastopore anlage then appears in its correct location dorsal in the gray crescent, this does not correspond with the tagging, which cannot be corrected. Second, it is possible that, in an egg held in a forced position, further rotation may have taken place after pressing; and in determining the location of the blastopore anlage, it is possible to be confused exactly by this slight rotation, so easily overlooked at the time of tagging. We must state, however, that our experiences indicate that these two sources of error seldom appear.

We arrived at the following conclusions which were based on the findings connected with the considerations presented at the beginning of this section. The dorsal region of the gray crescent is the location of the first blastopore anlage in a normal embryo. This location has special structure-forming capabilities. In the rotated embryo, the blastopore anlage is also usually found in the region where the dorsal part of the gray crescent had been. One may conclude from this that this region has maintained its special capabilities in spite of yolk shifting. This means that the important material of the gray crescent still lies at its original location in the rotated embryo. /399

In a majority of instances, however, the blastopore anlage is also located on the edge of a very white area. If it is not formed in the gray crescent, it is almost always connected to such very white matter. This leads to the conclusion that the formation of the blastopore anlage is triggered on the edge of very white material. If the white material is located outside the region of the gray crescent, then, in spite of the tendency toward blastopore formation on the part of the crescent, such formation is not always triggered there initially.

This leads to the final conclusion that, even in a normal embryo, the location of the gastrulation process is determined by the relationship between the gray crescent and white material on the surface.

The first anlage of the blastopore

Everywhere where the first blastopore anlage is observed, it always appears in the form of a fine, more or less branched, dark brown pigmented line. This is also the manner in which the dorsal blastopore lip appears in a normal embryo. This pigment line is formed usually as described above in a place where very white material meets less white material. It then deepens into a blastopore groove.

The development and typical forms of the blastopore

Seemingly normal blastopore. The blastopore groove was formed on the edge of a more or less roundish, very white area. Material may tuck in

over both lips at first. Somewhat later the extending groove gradually surrounds the very white complex and submerges it like a yolk plug, whereby light and also dark material in the vicinity is tucked over the lips of the more or less roundish blastopore. The yolk plug is grown over epibolically and the blastopore closes to a small slit.

Initially wide groove. The processes are generally the same. Since, however, the very white zone is narrow and long, the blastopore has the same appearance; i.e., it is a more or less long and therefore a nearly meridianal or parameridianal groove, the edges of which are separated to a greater or less degree by the yolk plug. This edge, which is finally completely enveloping, may be formed by the extension of a single groove or by the fact that a second, independently formed groove is formed on the opposite edge of the very white material. /400

Initially narrow groove which remains narrow. A straight pigment line, which deepens into a very narrow, fine groove is formed. Surface material is tucked in around its two edges. Smaller white areas impinged upon by the groove during its lengthening in the direction once embarked upon, which may be meridianal or parameridianal, are surrounded by the edges of the groove completely grown over and submerged between them like a yolk plug. The groove may reach various lengths. We designate a groove as long when it is longer than a half meridian. As in the case of Schultze's and Wetzel's circular furrow, it may extend around the entire embryo. Short grooves are those shorter than half a meridian.

The formation of a narrow groove from the onset is the most typical form of gastrulation in a rotated embryo. It shows the most pronounced deviation from the normal process of gastrulation and the further development into a duplication may be seen most easily with such a groove.

Which of the three above mentioned types of gastrulation forms appears is dependent entirely on the size of the very white mass lying on the vegetal surface and on its conformation. We found that the eggs of one and the same female have the same gastrulation form in a notable percentage of cases. This leads to the assumption that the sinking of the white yolk occurs with varying rapidity in different females, which is probably related to a variance in egg plasma viscosity and probably also directly to a variation in the amount of yolk present in the eggs.

In spite of the possibility of differentiating between these three types of gastrulation in rotated embryos, the individual variability is very great, so that the developments did not correspond exactly even in two embryos.

The closure of the blastopore

A "seemingly normal blastopore" is closed by means of concentric construction of its lips and the epibolic overgrowth of the yolk plug. An "initially wide groove" closes through the growing together of its edges and, therewith, the same epibolic growing over of the yolk plug to the narrow groove with its subsequent closure. The "initially narrow groove which remains narrow" disappears after the submergence of any smaller yolk plugs through the growing together of its edges, either from one end to the other or from both ends toward the middle. No epibolic overgrowth of larger complexes of white yolk occurs because this material has already almost completely entered the interior of the egg during the rearrangement process. The relationship of the remnant of the blastopore to the anus of the embryo anlage will be treated as thoroughly as is possible in the investigation by Miss Wittman. /401

The direction of the groove

In this section we shall discuss the direction of the groove in relation to the median plane and to the first furrows. We classify as grooves structures that are narrow from the beginning as well as those that are initially wide, even though the latter only slightly resemble grooves.

If a light band is present on the vegetal side of the blastula, the groove runs without exception in its longitudinal direction, either in the middle of the band or more along one of the edges. Since, however, smaller or larger very white complexes are usually present within the white bands and arranged along its longitudinal direction, the groove runs simultaneously along a very white streak or a number of very white areas. In cases where no band was developed, the very white areas are found in the irregularly formed lighter region of the vegetal surface, often either in the form of a pronounced very white streak or as a series of very white spots. In these instances the groove always lies in the direction of this streak or these spots.

The basis for this relationship is the influence of the very white material on the localization of the blastopore anlage, which we have already discussed.

The groove may run in a very arbitrary meridian or parallel to such a meridian. We have more or less completely described nine cases having the groove in a median position (Figures 6, 8, 10, 14, 17; as well as Plate VI, Figures 4, 7, 10 and Plate VII, Figure 17), seven cases having a frontal groove (Figures 7, 13, 15, 16, 24 and Plate VII, Figures 11 and 16), and twelve with an oblique groove (Figures 11, 18, 30, 31, 34-37, 39, 40 and Plate VII, Figures 13, 15). Some of these last figures show

an almost median groove position (Figure 18, for example) or grooves having an almost frontal position (Figure 30, for example). In other cases, the median plane cuts through the position of the groove at a 45° angle (Figure 36, for example). The pictures naturally also show many different positions, which are intermediate between the named extremes. The location of the groove with respect to the median plane thus varies greatly.

The groove also does not show any constant relationship to one of the first furrows and therefore cannot be said to lie only in the direction of the first or second furrow. /402 Based on the cases described in this and the next section, one may state that, in general, the groove runs perpendicular to the direction of the first furrow. This is true, for example, in eight of the twelve embryos having an oblique groove location, and for six of the seven embryos with the grooves having a frontal location, where the first furrow was median. Finally, this might be said of five of the nine embryos having a median groove, since the first furrow was frontal in these cases. We also see a deviation in relationships in four embryos with median grooves, one with a frontal groove, and in four having oblique grooves. We have therewith a total of 29 cases in which the groove runs perpendicular to the first furrow, and only nine cases, in contrast, in which this was not true. In general, the second furrow is perpendicular to the first. For this reason, one may determine that, in a great majority of the cases mentioned (19 of 28), the groove runs in the direction of the second furrow.

During the examinations conducted in the spawning periods of 1924 and 1925, we usually placed the eggs after pressing and rotation either in an icebox or in ice water. We also did this in one experiment during the 1926 spawning period. The eggs used in this experiment came from female VI. This cooling process caused the further cleavage processes to be considerably retarded. The results indicated that the groove occurred much more often in the direction of the first furrow than it did when cleavage was not retarded, irrespective of its position relative to the median plane. There are, therefore, connections of some kind between the location of the groove and the direction of the first furrow. It is not true, however, as Schultze and Wetzel maintained, that the groove invariably runs in the direction of the first furrow. Table VII gives more detailed information concerning this. The experiment tabulated here made use of the eggs of female VI (1926).

Some of the eggs (I) were rotated and placed in ice water at a time when the first furrow covered 1/3 to 1/2 of the surface of the embryo. The remaining eggs (II) were placed in ice water only after the furrow was completed. The table allows for the following determination. Forty-one of the eggs in group I showed the formation of a groove. The directions of 25 of these were parallel to that of the first furrow. Sixteen of these grooves had other locations. Of the 9 grooves which appeared

in the eggs of group II, none had a direction coincidental with that of the first furrow. In the first case, the first furrow formation was in process when the eggs were rotated. The material shifting proceeded for /403 this reason in such a manner that in a majority of cases the grooves had directions coincidental with that of the first furrow, which was being completed. In the second case, the furrow was already completed at the beginning of the shifting process. We see here that six of the nine grooves run in the direction of the second furrow, which was being formed at the onset of the rearrangement.

We were not able to ascertain any definite information concerning the other three cases. We did not pay any special attention to these cases. It could be that the directions of the three grooves were coincidental with that of the second furrow without being perpendicular to the first plane of cleavage. It is also possible that grooves run in the direction of one of the two third planes of cleavage, since the location of the third furrow in pressed embryos is not horizontal. This cleavage occurs in two meridian planes in such cases. In general, one may conclude that the groove runs in the direction of that furrow which is being formed at the time when the rearrangement of the egg substances begins.

Table 7. Cleavage in an Icebox (Eggs from Female VI 1926)

Stage at time of pressing, rotation and the beginning of cooling	No groove	Groove present			Total
		$\perp$ to first furrow	$\parallel$ to first furrow	oblique to first furrow	
I. First furrow 1/3—1/2 completed	42	5	25	11	83
		total 41			
II. Second furrow completed	5	6	0	3	14
		total 9			
Total	47	11	25	14	97
		total 50			

The localization of the capacity to form blastopore lips

Since, as we have mentioned, the first blastopore anlage tends to appear in the region of the original gray crescent, this crescent must

remain in place in spite of yolk rearrangement, and must retain the same especially pronounced tendency to form blastopores in the rotated egg as it has in the normal egg.

Since the first blastopore anlage may appear outside the region of the original gray crescent, these portions of the surface of the rotated embryo must not necessarily be capable of independent formation of blastopores. The formation of blastopores is triggered in these areas by the whitest material which comes into contact with them as a result of rearrangement of the yolk substances. /404

If we disregard the first beginning of blastopore formation, it is unquestionably true that any area on the surface of the rotated embryo is capable of becoming a blastopore lip. This follows from the following consideration. The blastopore groove can, as has been shown in many examples, more or less completely surround the embryo in, or parallel to, any arbitrary meridian. As a result of this, not only the region of the original gray crescent, but also any portion of the animal or vegetal field may become a blastopore lip, around which material is tucked into the interior. The determining factor as to which parts will form the lip depends on the initial direction of the groove.

This leads to the following conclusion. The localization of the first blastopore anlage is determined by the special capability of the region of the gray crescent, the influence of the white material meeting darker matter, or by a combination of these two factors. The direction of the first blastopore anlage then determines which additional portions of the surface will be used for the formation of the blastopore lips. The anlage obviously has an inducing influence on adjacent material in the direction once embarked upon, causing it to achieve an ability to tuck in, which it does not possess otherwise. This may definitely be compared with Spemann's (1918) findings on the uniting of equal-sided gastrula halves of Triton. The half dorsal blastopore lip, which contacts presumptive epidermis in this case, causes it to complement and complete the blastopore lip; i.e., to form a blastopore edge independently.

B. Gastrulation According to Section Series (Plates IX-XI)

This section will treat the sections made to show the processes occurring during gastrulation within the embryo. To make the widely varying conditions on the different embryos more easily understandable, we have included sketches of their surfaces. We have already done this in part in the previous section. The sketches will be used here to complement the previous ones and will be discussed in somewhat more detail. /405

It is clear that according to our findings concerning the structure of the blastula, which differ from those of Wetzel, his information concerning the inner processes of gastrulation (Part I, Section A) will also not be completely confirmed by our investigations. Furthermore, the investigation of the external processes of gastrulation showed that these may vary greatly, and that they definitely deviate widely from the normal gastrulation processes. In conformance with this, we may also expect to find varying deviations from the norm in our investigations of the inner processes of gastrulation in rotated embryos.

We feel that this point may be made clearer by presenting a schematic of normal gastrulation of Rana fusca in a sectional series in imitation of earlier authors (Figure 32). The sickle groove appears on median meridianal sections as a small, slit-like indentation on the surface, located on the border between the gray crescent and the vegetal white field. This corresponds to a shifting of the white yolk in the cleavage cavity toward the animal pole in the interior of the embryo. To the extent that the indentation deepens into the interior during the further development process, a wedge of white yolk extends opposite it into the cleavage cavity. The indentation in the interior remains narrow and slit-like up until the stage during which the blastopore becomes annular on the exterior of the embryo. The outer boundary of the blastopore appears on the section series as an enveloping fold, and one has the impression that an invagination process has caused the material of the marginal areas to penetrate the interior in the form of a ring-like wall which pushes some of the white yolk in front of it and pulls some of it with it into the interior. Simultaneously, the dorsal and lateral portions of this marginal zone are pushed externally over the white yolk.

According to the idea of Goette, whose description is followed in this section, the primitive gut slit expands in the interior, causing the lumen of the cleavage cavity to be suppressed to a greater degree by the white yolk. Goette considers this suppression to be complete, so that the original cleavage cavity is taken up completely by white yolk or by the archenteric cavity. This cleavage cavity often remains in part according to Schultze. Meek states that it remains in its entirety and then fuses with the smaller archenteric cavity to form a common cavity (see Part VI, 2).

The dorsal boundary of the primitive gut, formed by invagination of marginal zone material, is thin and represents the roof of the primitive gut. The ventral boundary (the floor of the primitive gut) is thick and is formed from the invaginated and overgrown white yolk cells. The roof of the primitive gut contains three anlagen (the dorsal intestinal epithelium, the chorda, and the mesoderm band) which, according to O. Hertwig, forms as a solid cell proliferation lateral to the chorda anlage and then pushes itself in a ventral direction between the ectoderm and the yolk. Other authors maintain that the mesoderm band is

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located only partially in the roof of the primitive gut. Above all, the ventral portion must be split off relatively late from the mass of yolk cells.

If one applies to Rana the more recent opinions concerning the gastrulation process, especially those expressed by Vogt (1922c, 1923b) derived from Triton, the following picture is obtained; this agrees in many respects with the earlier opinion of O. Hertwig (1906).

1. The externally visible white yolk of the blastula (the vegetal field) sinks into the interior of the embryo and thereby forms an open groove running dorsally.

2. The region of the gray crescent, which corresponds to the marginal area of Triton, tucks in around the blastopore lips; preponderantly, the dorsal lip. It then lies under the external surface, closes the groove formed by the white yolk dorsally to form the roof of the primitive gut, and then hangs down as a mesoderm over the side of the white yolk.

3. During the sinking process, the white yolk is also epibolically overgrown by the edge of the animal field; that is, by the tucked in marginal zone.

4. The archenteric cavity (the groove of the sunken vegetal field) is separated dorsally from the roof of the primitive gut, which contains only the notochord anlage.

1. The Beginning of Gastrulation

At first we shall treat only the initial stages of gastrulation, whereby we shall confine ourselves principally to the relative positions of the blastopore slit, the white yolk, and the cleavage cavities.

a. Embryos having no cleavage cavity or only one

In this section, we shall discuss four embryos from the same female, which were fixed in the beginning of the gastrulation stage, and in which section studies showed only one cleavage cavity each in three cases, and no cavity in the fourth instance. Furthermore, a fifth embryo with one cleavage cavity will be discussed here because it exhibits an important pecularity with respect to the onset of its gastrulation. /407

In an earlier portion of this study (Plate IX, Figure 29) we observed a meridianal section which showed a relatively good separation of light material in the middle and two lateral complexes of dark yolk

cells. We mentioned at the time that no cleavage cavity could be found on this embryo. A distinct white band was visible on the outside, which was perpendicular to the frontally located first furrow and therefore stretched medially across the vegetal surface (Figure 17). A streak of pure white material extended down the middle of the white band.

In the dorsal region, approximately in the middle of the region of the gray crescent, somewhat to the left in the white band and on the left boundary of the very white streak, the first beginning of the groove could be seen shortly before the embryo was fixed. Figure 29 (Plate XI) shows the groove in a section perpendicular to its longitudinal direction. One sees that the groove cuts in rather shallowly and somewhat radial at first on one side of the white yolk between it and the dark material on the left. We may therefore determine without question that the gastrulation began entirely on the surface as in a normal embryo, in that a slit appeared in the dorsal region on the boundary between light and dark surface material. This slit deviates from the norm only in its direction. This deviation was caused by the corresponding abnormal arrangement of pure white material on the surface.

In the three remaining embryos the same determination could be made even though they had cleavage cavities in the interior, and even though these cavities had different locations. In Embryo XII 7 (Plate IX, Figure 30), this cavity was located on the right side of the white yolk. The first furrow on this embryo was oblique and, as it was fixed, a long oblique groove in the middle of a white band running in the same direction had formed (Figure 18). The section shown from this series is taken from the middle, and passes therefore approximately through the middle region of the groove. It is to be noted here that the groove pushes the white yolk matter somewhat into the laterally located cleavage cavity. In other words, the groove is in the region of the white yolk cells, but it is rather close to the cleavage cavity.

If one considers the cleavage cavity to be in the zone of white yolk, one may state that the groove cuts in approximately in the middle of this zone in the longitudinal direction of the white band, which is visible on the surface. This opinion is substantiated by examination of the entire series, especially of the sections through the dorsal end of /408
the groove where the cleavage cavity no longer appears. A study of the entire series shows further that the groove is shallower on the ventral end, but that on the other hand it begins to deepen as one follows the sections toward the dorsal end. At the dorsal end itself, the groove is again shallower. The groove therefore cuts deepest at about the point where the gray crescent material originally bordered on the vegetal field. It is very probable that the groove had its first beginnings at this location. The reason why we present a section from this particular embryo lies in the relative position of the slit and the single

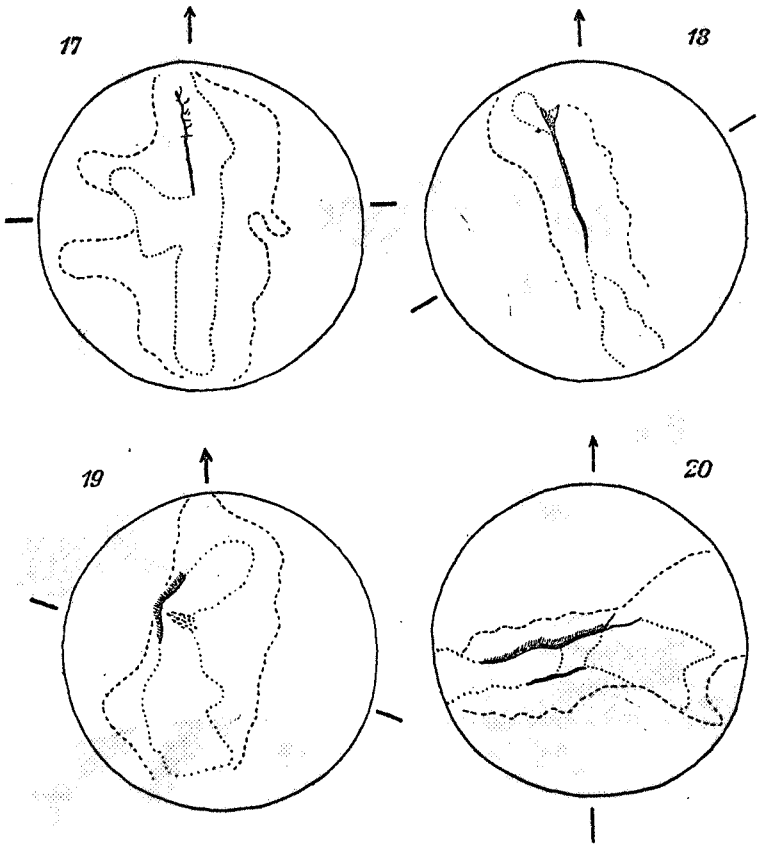


Figure 17. Embryo XII 5, vegetal side; surface view of section, Plate IX, Figure 29.
Figure 18. Embryo XII 7, vegetal side; surface view of section, Plate IX, Figure 30.
Figure 19. Embryo XII 8, vegetal side; surface view of section, Plate IX, Figure 31.
Figure 20. Embryo XII 9, vegetal side; surface view of section, Plate IX, Figure 32.

cleavage cavity present. The groove lies in the vicinity of the cavity, /409
which is located on one side of the white yolk.

An exactly reversed relative position in the interior may be found in the following case (Plate IX, Figure 31). In this embryo, the first furrow was oblique on the blastula. A white band was perpendicular to this furrow, and therefore also oblique. This band had a very white streak running down its center in a longitudinal direction (Figure 19). On the left edge of this light material and somewhat closer to the dorsal than to the ventral region, a short, approximately median groove was

present at the time of fixation. Figure 31 (Plate IX) shows a cross section of this groove. It cut into the interior on the extreme left side of the white yolk, as may be seen in Figure 19, whereby the cleavage cavity was located directly opposite to it on the right side.

Another relative position may be found in Figure 32 (Plate IX). The first furrow ran in a median direction on this embryo. The white band on the blastula was frontal (Figure 20). When the embryo was fixed, it had a long, frontal groove on the dorsal edge of a very white streak in the white band. This streak was interrupted in the middle. A second groove-like fold, which was much shorter, was located on the ventral edge of the very light material. This picture was observed in many instances. It usually changed during the further stages of development to the point that the very white material was submerged like a plug, after which the two "grooves" closed over it into a unitary blastopore slit, over the two lips of which the surface material would then tuck into the interior. This was described in detail in Part IV, Section A.

The section presented was cut somewhat to the right of the median plane through the embryo. As a result of this, only the long, dorsal groove was encountered. It sinks into the interior at a point dorsal from the white yolk complex. The one cleavage cavity in this embryo lies somewhat diagonally on the dorsal-animal portion of the white yolk, so that the groove seems to be moving toward it. The groove has not yet, however, cut very deeply into the interior of the embryo.

The fact that, aside from the occurrence of a blastopore slit dorsal to a very white complex, a gastrulation lip could also form on its ventral boundary, which would be independent of the first slit, is illustrated to the extreme in Embryo IX 15 (Figure 21). The first furrow was oblique, approximately midway between median and frontal. No white band appeared on this embryo, but in the beginning stages of gastrulation and /410
at the time of fixation, a large complex of gray-white material stretched approximately perpendicular to the first furrow in a wide band from left-ventral to right-dorsal across the vegetal field. This wide band had a very light zone in the middle, which stretched longitudinally in about the direction of the first furrow.

In principle, these represent the same conditions present in the embryos just discussed. On the left-dorsal and right-ventral edge respectively of this band-like complex, where the very white material bordered in the dark surface of the embryo, an oblique gastrulation slit appeared. Figure 33 (Plate IX) shows a middle section of the series which was cut perpendicular to the two slits.

The section series indicates clearly that each slit represents the /411
beginning of a primitive gut formation which is very similar to a normal primitive gut in the beginning stage of development. Cellular material

from the dark surface of the embryo has obviously rolled over both grooves into the interior. This cellular material pushes past the white yolk in the animal direction. It should also be noticed that the cleavage cavity on this embryo, which is stretched in a vegetal-animal direction, runs through the middle of the white yolk, and that the two widely separated gastrulation slits on both sides of the cleavage cavity has pushed ahead of it and the white yolk into the interior.

Summary. In agreement with the information furnished above (Part IV, Section A.7) concerning the location of the first point of origin of the groove, we were again able to determine from our examinations described here that gastrulation had its beginnings in the region of the gray crescent. This is true, completely independent of the presence or lack of a cleavage cavity in the interior of the embryo or of the location of such cavities in those embryos having them. This may easily be seen from the surface sketches of Embryos XII 5 and XII 8 (Figures 17 and 19). Embryo XII 7 (Figure 18) yields an insight into the entire series, since the groove is deeper on the dorsal than on the ventral side, and this is a sufficient indication that the gastrulation began there. In Embryo XII 9 (Figure 20), the gastrulation surely began first in an area dorsal from the very white streak. We may further assume, in analogy to other embryos, that it first began in the left horn of the crescent. We also observed the appearance of a groove dorsal on the gray crescent in Embryo IX 15 (Figure 21).

With the exception of one embryo (Plate IX, Figure 29), in which no cleavage cavity could be determined, the locations of cleavage cavities and gastrulation slits relative to one another varies greatly. In one case the groove cuts into the interior in the vicinity of the cleavage cavity (Plate IX, Figure 30). In another case it cuts in somewhat farther away from the cavity (Plate IX, Figure 31). In a third instance it cuts into the middle of the cleavage cavity (Plate IX, Figure 32). With respect to this last case, we must add that the slit, which made a delayed appearance ventral from the very white material and which was not observed in further sections, cut into the middle of the white yolk, although it is very flat at first. On the other hand, the two independent slits in another case (Figure 21 and Plate IX, Figure 33) cut into the interior past the white yolk on both sides. /412

The arrangement of the white yolk with respect to the cleavage cavity also varies greatly in these embryos. This has already been discussed in another regard above.

b. Embryos with two cleavage cavities

The arrangement of the different components of gastrulation relative to one another is also quite varied in those embryos having two

cleavage cavities. Aside from the number of cleavage cavities, Embryo IX 14 (Figure 22 and Plate IX, Figure 34) is very similar to Embryo IX 15, which had one cavity and which, like IX 14, came from the same female. In it, (Embryo IX 15), a very typical white band was located on the blastula and was directed frontally. In the middle of this band, a somewhat elongated streak of very white material stretched from left to right. Gastrulation began in the form of an already rather long groove at the point where the streak bordered on the darker portion of the band. Later, a small depression appeared on the ventral edge of the very white zone. The section (Figure 34) which was cut almost exactly median through the gastrula shows first that a relatively good separation exists between the middle region of white yolk and a covering of darker material on this embryo. Furthermore, two cleavage cavities are present: a very large one, ventral from the white yolk between it and the dark portion of the egg, and a second, smaller cavity, located approximately in the middle of the white yolk itself; i.e., more to the dorsal side than the larger one. The dorsal lip of the blastopore cuts into the interior of the embryo at a point dorsal from this cleavage cavity, passing both this cavity and the white yolk. It may be clearly seen that dark surface material had tucked in over the lip here and, directed dorsally, the roof of a primitive gut was developing.

The ventral gap may be seen in its first beginnings. It points directly into the large cleavage cavity located there. According to our experiences with other embryos, this ventral groove would have probably forced its way between the ventral dark material and the large cleavage cavity in the course of the further sinking process. After the white yolk had been submerged, this groove would have simultaneously merged with the dorsal slit into one groove; this was described in Part IV, Section A. What interested us here was the determination that two separate slits were formed in the presence of two cleavage cavities, in /413 contrast to the two cases described above which included only one cleavage cavity. In this case, the dorsal slit was again certainly formed much earlier than the ventral, and was at a more advanced stage of development at this point.

Another embryo with two cavities, Embryo X 19, shows approximately the same conditions as the previous embryo with respect to the cleavage cavities. The difference is that the smaller cavity was located to the right, and the larger one to the left in the white material. In the blastula stage, the embryo had an arrangement of lighter material on the vegetal surface, which could not be designated as a typical form of band (Figure 23). In general, the lighter portions of the surface were extended lengthwise in a somewhat oblique direction to the median plane. A very white complex was located on the dorsal left in this band, and the arched gastrulation slit was formed there on the dorsal edge of this zone and opened in a ventral direction. When the embryo was fixed, it presented an almost normal picture of gastrulation, with the exception

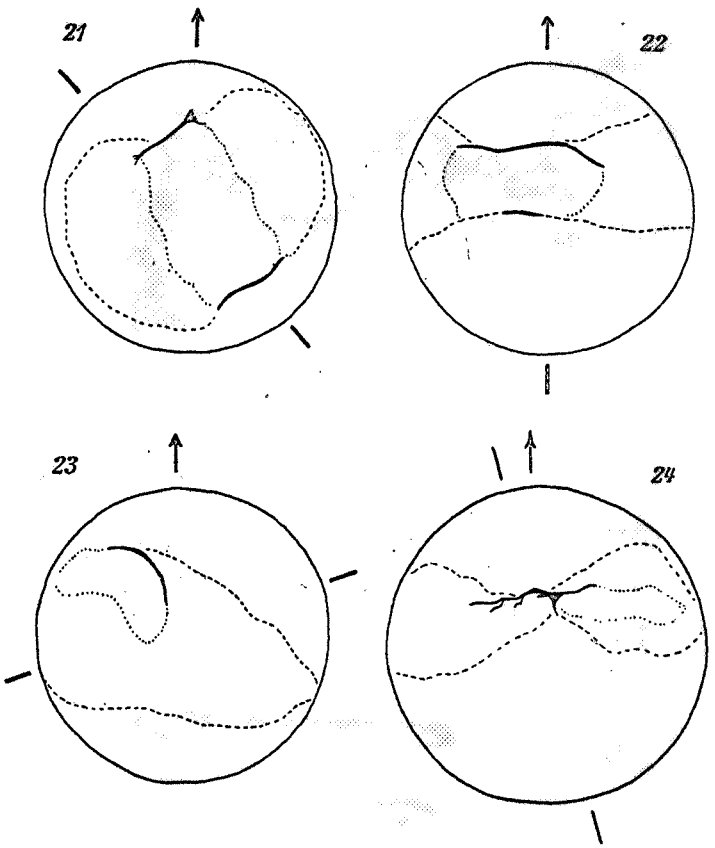


Figure 21. Embryo IX 15, vegetal side; surface view of section, Plate IX, Figure 33.
Figure 22. Embryo IX 14, vegetal side; surface view of section, Plate IX, Figure 34.
Figure 23. Embryo X 19, vegetal side; surface view of section, Plate IX, Figure 35.
Figure 24. Embryo XII 6, vegetal side; surface view of section, Plate IX, Figure 36.

of the location of the blastopore. The sections, which were cut perpendicular to this arcuate line and somewhat oblique to the median plane through the embryo, show two cleavage cavities (Plate IX, Figure 35). As we have already indicated, the smaller of these lies ventral from the white yolk and separates it sharply from the dark material. The second, much larger cavity is located on the dorsal side of the white yolk, but also penetrates deeply into the middle of it. The gastrulation slit pushes forward with some of the white yolk in front of it laterally in the direction of the dorsal cleavage cavity. The tucking-in of surface

material against the dorsal side, and therewith the formation of the roof of a primitive gut is in progress.

A third embryo with two cleavage cavities (XII 6), which we shall discuss briefly, presents an entirely different relative position of its individual components. It is the only embryo in which we found conditions approximating those described by Wetzel. The embryo had a rather frontally directed, typical white band with a very white longitudinal streak in its right half (Figure 24). As usual, the gastrulation occurred in the form of a frontal groove at the point where this streak bordered on darker material. The section (Plate IX, Figure 36) shows that the groove cuts into the white yolk at a point approximately midway between the two cleavage cavities. These two cleavage cavities are dorsal and ventral, respectively, from the white yolk and do not separate it very sharply from the dark yolk.

Summary. We were able to redetermine in these three embryos, which had two cleavage cavities each, that the onset of gastrulation occurred at about the normal place and was also related to very white material on the surface. On the other hand, the location of the cleavage cavities in the interior had absolutely no influence on the point of origin of gastrulation. The groove cannot only, as Wetzel stated, cut through at a point between the two cavities, it may cut in laterally from them so that both cavities are located on the same side of this groove. This series showed very clearly that the first groove anlage has no relationship whatsoever with the cleavage cavities. /414

c. Three cleavage cavities

Embryo XIII 4 will be discussed briefly as an example of three cleavage cavities and a beginning blastopore slit. The surface sketch (Figure 35) of the beginning gastrula shows an irregularly bounded grayish-white field on the vegetal side, which contains a very white zone in the dorsal area. The gastrulation has begun there on the boundary of the grayish-white field in the form of a shallow depression. The section (Plate 10, Figure 38) which was cut about frontally through the area of this depression, shows three cleavage cavities whose relationship to the materials of the egg has already been discussed in detail (page 52). Most importantly, one recognizes on this section that the area where gastrulation began certainly could not have been determined by the location of the cleavage cavities. The blastopore depression cuts into the embryo to the right of the vegetal cleavage cavity on the right side of the white yolk. It is directed toward the right cleavage cavity.

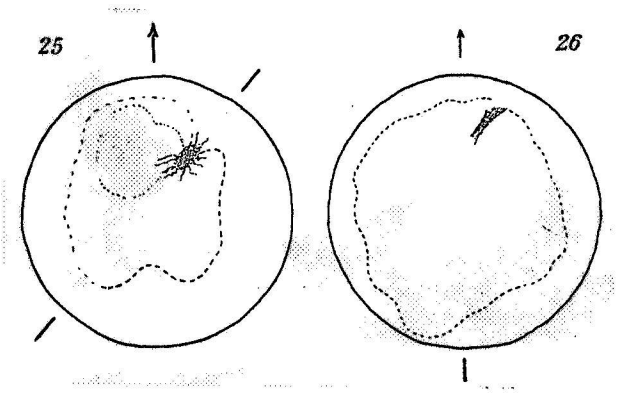


Figure 25. Embryo XIII 4, vegetal side; surface view of section, Plate X, Figure 38.
Figure 26. Embryo XIII 5, vegetal side; surface view of section, Plate IX, Figure 37a-c

d. Four cleavage cavities /415

The following may be said about Embryo XIII 5 (Figure 26 and Plate IX, Figure 37a-c), which we present as an example of four cleavage cavities. It assumes in general the same position as Embryo XII 6 (Plate IX, Figure 36) with respect to the two cleavage cavities determined there, if we regard the cavities located on the same side of the white yolk in the present case as one cleavage cavity. The location of the gastrulation slit is also similar in that it cuts between two cleavage cavities in the dorsal region. The relative positions do not completely conform with one another however (Plate IX, Figure 37a). In this case, the cleavage cavities are located to the right and left of the white yolk, whereas they were located dorsal and ventral in the first instance. Also, no white band was observed in this embryo, but only an irregularly outlined, grayish-white vegetal field (Figure 26), in which the blastopore beginning occurred dorsally in the gray crescent. As we have so often done in the past, we must determine here that a certain relationship exists between the location of the gray crescent and the place where gastrulation begins. We must also determine that the blastopore lip is independent of the material dispersion in the interior of the embryo.

e. Five cleavage cavities

Finally, we shall discuss still one more final case of beginning gastrulation (XIII 6), because we found five cleavage cavities in this embryo. Superficially, the picture presented at the time of fixation was similar to that of the others, with the exceptions that a small, very white complex was present in the vegetal field and that the

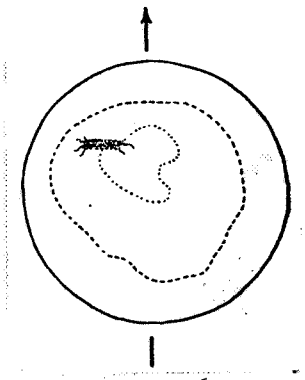


Figure 27. Embryo XIII 6, vegetal side; surface view of section, Plate X, Figure 39a-b

blastopore anlage was more to the left in the gray crescent than was the case with the others (Figure 27). We have already stated above all that is necessary concerning the locations of the cleavage cavities within the embryo (Plate X, Figure 39, a and b). They correspond generally to the locations of the four cavities described earlier. The gastrulation slit cuts, in the same manner, between two cleavage cavities in the dorsal region into the white yolk (the small depression E, located to the left of the blastopore, was not noticed on the surface view). In this case, therefore, we have the same relationship between the first occurrence of the blastopore slit and the gray crescent. We may also determine once more that no relationship exists between the location of the blastopore lips and the material dispersion in the interior of the embryo. /416

f. Results

It may be said, concerning all cases of beginning gastrulation, that the location of the first appearance of the groove is certainly completely independent of the number and location of the cleavage cavities in the interior of the embryo, which may vary widely. Gastrulation, on the other hand, almost always begins in the region of the gray crescent and almost regularly on the dorsal boundary between a very white complex and darker parts of the egg. A gastrulation slit completely independent of the dorsal one may appear on the ventral boundary between this very white complex and the dark surface of the egg in some instances. When this occurs, however, it is usually later in respect to the former slit. As we have already seen (Part IV, Section A), both slits approach one another during the further stages of development, enclose the light complex like a yolk plug, and combine after the submergence of this plug

into an outwardly unitary groove, about whose lips material then tucks over into the interior. The longitudinal extension of this groove, whether it occurred initially as a narrow groove or was formed subsequently from two separate anlagen, always moves in the direction of the longitudinal extension of the very white material or of the white band on the vegetal surface.

We demonstrated above that the white yolk in the interior of the embryo by no means extends itself in any particular meridianal direction as a result of rotation, even when a white band appears on the surface. As a result of this, the arrangement of the very white material on the surface does not necessarily correspond to a like arrangement of the white yolk in the interior. Only in one case did we observe, in conformance with the direction of the white band on the surface, the white yolk in the interior having a greater length in the direction of this band than it had perpendicular to it (Plate VIII, Figure 26a).

We observed one case in which the light portions of the surface did have a greater extension in a certain direction, where, in its longitudinal direction of extension, a groove was formed which enclosed this material like a plug between its lips. The sectional examination of this embryo showed that the white yolk in the interior, exactly perpendicular to the longitudinal direction of the surface white yolk, was extended somewhat further (Plate X, Figure 40). The longitudinal extension of the groove is therefore dependent only on the longitudinal extension of the white yolk cells lying on the surface, but not on the yolk arrangement in the interior of the egg. /417

The gastrulation of rotated embryos certainly does not begin, as Wetzel proposed, to a certain extent in the interior of the embryo between the first two cleavage cells in the white yolk (see Figure 2). On the contrary, the onset of gastrulation occurs on the surface, normally in the form of a pigment line on the edge between very white and dark material of the vegetal surface, generally in the dorsal region. The pigment line subsequently deepens into a slit which penetrates into the interior. With respect to the internal processes, which then begin operating in the interior, we are able to differentiate between two major types of gastrulation onset.

1. Exactly or approximately at the normal place, a narrow, initially more or less long groove is formed. This groove is relatively straight in contrast to the more normal arcuate gastrulation slit. A pigment line usually appears as the first anlage of this slit. It may also appear as a small pigmented depression. In all the development stages studied on the slides mentioned above, this groove is only recognizable as a slit which is initially rather shallow and penetrates more or less radially into the interior without making any significant inroads

into the compact white yolk. The slit is simple (not forked) and quite narrow at the deepest part. In many cases, it may only be differentiated through the pigmentation of the cells bordering it. It appears to be somewhat widened on the surface. In the interior of the embryo, therefore, it is at first very similar to a normal dorsal blastopore slit at the beginning of gastrulation, with the exception that it penetrates approximately radially into the interior. This type of blastopore formation is nothing other than the occurrence of a "groove which is initially narrow."

2. A blastopore anlage is formed, which externally resembles a normal gastrulation process more than the first type, in that a yolk plug formation is under way. This process may occur in two different ways. First, an arcuate blastopore appears at the outset along a roundish complex of very white material on the vegetal surface. It closes during further stages of development into a more or less roundish blastopore which surrounds a yolk plug. This corresponds to the formation of a "seemingly normal blastopore" from one anlage. The other possibility is that a straight slit is initially formed at the normal place, /418
dorsal from a very white streak on the surface. This is similar to the first case: a groove. Subsequently, supplementing this first blastopore slit, a second, independent slit appears ventral from the very white complex of material. Both slits extend themselves along this complex until their ends meet and a yolk plug-like formation is also enclosed between them. This corresponds to the formation of a "seemingly normal blastopore" or a "wide groove" from two anlagen.

Of this second type of gastrulation, we have only discussed those embryos with sections in which the blastopore slit initially does not cut very deeply into the interior and is quite narrow in the interior.

Both types of gastrulation deviate from the normal blastopore formation. A purely external observation would be sufficient to make this clear in the case of groove formation, since an arcuate blastopore, not a straight one, occurs at the outset in a normal gastrulation process. Furthermore, the section study shows that the slit does not penetrate obliquely into the interior, but rather radially. These plug formations mentioned are also abnormal since in those cases where, for example, a ventral slit appears alongside the dorsal blastopore slit, it is formed much earlier than a normal ventral blastopore lip. As a result of this, the tucking of material around this lip may begin earlier than normally is the case. Furthermore, the white material is enclosed by a blastopore lip at two different locations for a time, which never occurs in normal gastrulation. Even when the blastopore slit appears initially as an arch, this is never a normal process with respect to form or location. Most importantly, this arcuate blastopore closes itself much more quickly into a circular one, so that, here again, on the ventral side gastrulation begins much earlier than in a normal embryo.

2. Further Course of Gastrulation

We can, therefore, differentiate between two types of gastrulation in our embryos. First, we have gastrulation during plug formation, which occurs in embryos still having rather large complexes of pure white material on the vegetal side. This material is then submerged like a yolk plug in the roundish blastopore or the wide groove. In this type, therefore, the partial process of gastrulation, consisting of the submergence of the white yolk, must be carried out to a large degree during gastrulation, since the white yolk has not been completely submerged during cleavage. As a result of this, the process of the epibolic growing over of the white yolk is also accomplished during gastrulation insofar as this very white matter still lies on the surface. This process occurs in the manner discussed, in that the lips of the blastopore thrust together over the white material. /419

The second type of gastrulation is that without plug formation. In this second type, the process of submergence of the white yolk is almost completely missing, since it has already sunken during cleavage and has not left larger complexes on the vegetal surface. Consequently, the process of epibolic growing over is also missing from this second type of gastrulation. In spite of this, gastrulation in the case of an initially narrow groove also has more or less large yolk plugs; i.e., also residues of white yolk on the vegetal surface, with resulting submergence and epibolic growing over. The yolk plugs in this type are relatively small, however. One may say, though, that in cases of initially narrow grooves whose lips enclose a yolk plug, the gastrulation processes are the same as with an initially wide groove. When we refer to gastrulation with the formation of an initially narrow groove in the following portions of this study, we are referring to those areas where no yolk plug is present.

a. Gastrulation with the formation of a yolk plug

On Embryo IX 19 (Figure 28) there is a very white complex in the middle of the somewhat frontal light band, which is bounded dorsally by a blastopore groove. A thinner, interrupted groove-like depression (E) was formed later on the ventral edge of the very white material. Furthermore, a second blastopore groove, which was unquestionably a second blastopore anlage, was formed on the ventral edge of the light band. Clearly, a blastopore in the form of an initially very wide groove had been formed from this, and this blastopore had enclosed the very white material and portions of the light band as a yolk plug.

The embryo was fixed two hours after the stage of development described. Plate X, Figure 41 shows it in an approximate median section. A very narrow slit cuts deep into the interior dorsal from the white yolk.

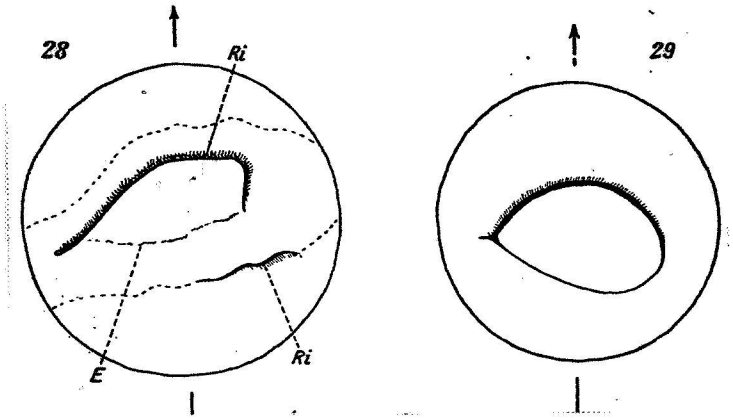


Figure 28. Embryo IX 19, vegetal side; surface view of section, Plate X, Figure 41.
E, depression; Ri, blastopore groove.
Figure 29. Embryo X 20, vegetal side; surface view of section, Plate X, Figure 42a-b

Directed outward and toward the dorsal side, the slit is bounded by small, darkly pigmented cells, which have obviously been tucked over the lips from the surface. They form the roof of a primitive gut which cannot, /420
however, be exactly differentiated from the ectoderm lying on top of it. Unmistakably recognizable white yolk cells are located ventral from the slit; these form the floor of the gastrulation cavity. Ventral from the slit, the yolk plug protrudes toward the outside. On the section, the plug shows a definite indentation in the middle, located exactly on the boundary between very white yolk material and the less white portion of the light band (E in Figure 41, Plate X, which may be compared with E in Figure 28). From the sequence of the entire section series, it is evident that the slit on this embryo, ventral from the very white streak, does not represent a folded over gastrulation lip, but rather the still further ventrally situated (Ri) blastopore groove.

The latter is recognized primarily in the portion which lies mostly on the right (not shown in the section), where it penetrates most deeply into the interior and probably first developed there. It may, however, be recognized to some degree on this section.

In a normal embryo with a dorsal blastopore slit of even depth, such as the one now being discussed, a rather large plug of white yolk cells penetrates into the cleavage cavity in the prolongation of the slit toward the interior. In other words, it would be recognizable that, due to gastrulation, the yolk is pushed into the cleavage cavity. In this case, one cannot see such a development. On the contrary, the sinking

dorsal groove (i.e., the gastrulation cavity) has enclosed the yolk and the dorsal cleavage cavity from the outside and merely separated it from the surface material. This deviation from Goette's description of normal /421 gastrulation corresponds closely with the description furnished by Meek (1923) (see Part VI).

At the time of fixation, the embryo in Figure 29 already showed a well-outlined yolk plug, which was enclosed by a blastopore lip which was frontally somewhat elongated and otherwise approximately roundish. We do not know just how this blastopore was formed. It is possible that it was formed from a blastopore anlage similar to the one in Figure 23. The embryo was cut parallel to the median plane. Figure 42a (Plate X) from the series shows a section rather far to the right of the median plane, while Figure 42b shows one located somewhat left of it. It shows that on the whole periphery of the plug, the outer edge constitutes a true gastrulation lip, over which significant portions of the surface have already tucked in to the interior. The greatest amount of tucking obviously took place around the dorsal lip, while only very little material had tucked over the ventral lip. The submerging blastopore groove had not invaginated the yolk into the cleavage cavity either dorsally or ventrally.

The gastrulation cavity of this embryo deviates from all the others in that it is not a narrow slit, but is rather unusually widened (Figure 42b). It is separated from one cleavage cavity only by a thin lamella of white yolk cells, which is interrupted at several places, so that the gastrula cavity and one cleavage cavity merge and form a common cavity, exactly as it may occur during the development of a normal embryo. Otherwise, the white yolk cells bounding the gastrula cavity form a very good tessellated epithelium. The dorsal gastrulation cavity is now covered (Figure 42a) toward the outside only on its smaller right half by a roof of a primitive gut which is well defined by the ectoderm. In this region, the separated roof of the primitive gut extends very deeply into the interior, almost reaching the animal pole, whereas in the area of the median plane and in the entire left half of the embryo, the primitive gut roof is not separated from the outer surface of the embryo (Figure 42b). Along the entire ventral blastopore lip one recognizes a well-separated, rather thick complex of small, rather darkly pigmented cells. This complex does not reach very deep onto the embryo, but one can hardly identify it as the material complex, which is ordinarily tucked around the normal ventral blastopore lip.

The embryo in Plate X, Figure 43 also formed a yolk plug in a manner unknown to us. The section first shows very well that gastrulation does not occur into the cleavage cavity, but rather it encloses the yolk plug, separating it from the surface material. The form and arrangement of /422 the yolk cells gives one the impression that the yolk plug is dragged into the depth along with the surface material rolling over the lips.

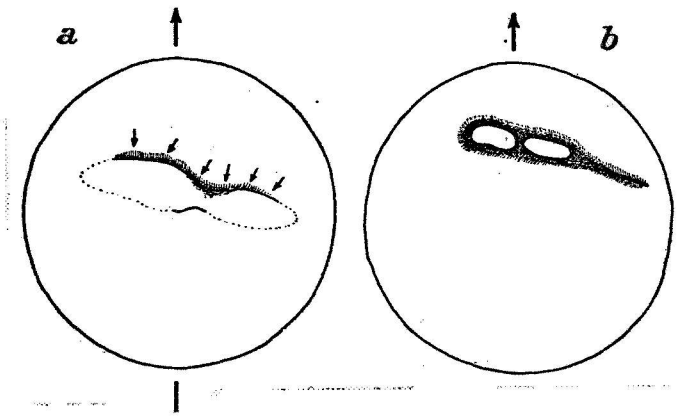


Figure 30. Embryo VII 6, vegetal side. a, 5:15 p.m.; b, 9:30 p.m. Surface view of section, Plate X, Figure 44

This is only possible if the yolk cells are dammed up in the interior, which seems to be the case. According to the section, this embryo also has on the dorsal side a well-defined primitive gut roof extending far into the depths, and on the ventral side another that is only a little shorter, so that one could not compare it to the conditions present in a normal ventral blastopore lip.

In Embryo VII 6 (Figure 30), a groove also occurred first at the beginning of gastrulation dorsal from the very white complex. At first it extended in the form of a simple groove on the vegetal side along the very white streak. About six hours after the beginning of gastrulation, a slight depression appeared on the ventral boundary of this streak (Figure 30a). This depression gradually merged with the dorsal slit until, at the time of fixation, a wide groove was formed (Figure 30b). It enclosed two deep-lying plugs between its lips. This agrees with the wide slit shown on the sections (Plate X, Figure 44), which runs from the surface into the interior and forks in the depths into two branches over the white yolk, a long dorsal branch and a somewhat shorter ventral branch.

The epiboly of the white yolk on this embryo was therefore not quite completed. No definitely defined primitive gut roof could be recognized above the longer dorsal gastrulation branch, but it is bounded in the dorsal direction by small, darkly pigmented cells, which obviously have been tucked over the lips from the surface. The short ventral blastopore branch is covered by a rather short, but nonetheless, typical primitive gut roof, which gives the same impression as the first primitive gut anlage above the normal dorsal blastopore lip. /423

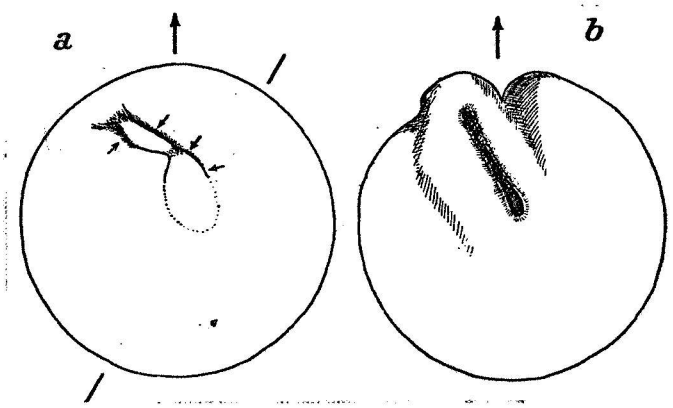


Figure 31. Embryo VII 5, vegetal side. a, 5:45 p.m.; b, 9:30 p.m. Surface view of section, Plate X, Figure 45

In Embryo VII 5, an oblique slit was formed at first dorsal from a very white complex in the white band which, ventral to the white band, was associated with a second slit (Figure 31a). This second slit was then extended along the entire ventral edge of the very white streak, and both slits met at its lateral ends and formed an oval, closed blastopore which submerged all the white material like a yolk plug in that the dorsal and ventral edges approached each other (Figure 31b). The yolk plug in this type of gastrulation, just as was true in all the other types discussed, must still have been epibolically overgrown to a certain degree. In agreement with this behavior, the section series shows that the blastopore slit in the interior is forked perpendicular to its longitudinal direction (Plate X, Figure 45). Of these two fork branches, the dorsal-right was the first formed and is much longer than the ventral-left slit.

Both slits are covered toward the outside by a typical primitive gut roof, and enclosed on the interior side by a similarly typical primitive gut floor. Floor and roof are bounded by quite different cells, some of which are small and pigmented, while others are large and loaded with large yolk granules. The two primitive gut roofs are opposed in the interior of the embryo, inserted between the outer surface and the white yolk. It is unquestionably true that a true primitive gut roof also is present over the ventral slit in this embryo. One also sees here once again that the gastrulation did not ensue into the cleavage cavity. /424

In this embryo, a shallow furrow was formed on both sides of the groove. These furrows became deep indentations on the dorsal edge by the time the embryo was fixed (Figure 31b). Since this embryo was sectioned exactly perpendicular to the direction of the groove, these indentations

do not appear in this section, which was taken from the middle portion of the series. Figure 45 (Plate X) shows only a very shallow indentation of the surface located some distance away from the gastrulation slit and on both sides of it. A corresponding elevation of the groove lips may also be seen.

In rotated embryos initially presenting an arched blastopore slit on the dorsal boundary of the very white complex on the vegetal surface, complete plug formation does not necessarily occur very early through rapid closure of the blastopore into a roundish structure. Rather, the blastopore may remain for a long time as a ventrally open groove. In this case, the gastrulation process naturally makes an exceptionally normal impression, with the possible exception of the often abnormal location of the blastopore. Sections from such embryos, which are cut perpendicular to the blastopore, also show seemingly normal aspects (Plate X, Figure 46). This section was cut from an embryo fixed six hours after the beginning of gastrulation and had, at that time, a horseshoe-shaped, curved blastopore. The section shows normal conditions except for the location and size of the cleavage cavity and with the exception that the white yolk has, to a large extent, shifted during cleavage into the animal region of the embryo. As a result of this, there is no evidence of yolk invagination on the section, which would have to be shown on a section cut through a normal embryo. Naturally, the primitive gut roof is always formed in one direction in such cases. In the case being discussed, it is only slightly differentiated from the upper surface.

Summary. Gastrulation in rotated embryos with the formation of a yolk plug is similar in many respects to normal gastrulation. In other respects it differs greatly from the norm. This may be seen in the following schematic summary of the results of this section.

A gastrula having a normal beginning is shown in a median section /425
in Figure 32a. One sees the cleavage cavity in the interior of the embryo and the dorsal blastopore lip, which is just forming, on the dorsal edge of the white field. Figure 32b shows how a large amount of surface material tucks in over the dorsal blastopore lip, but hardly any over the ventral lip, so that the primitive gut roof forms only dorsally and not ventrally. The yolk is pushed in the form of a wedge into the cleavage cavity as a result of the tucking of material around the dorsal blastopore lip. The white yolk fills the cleavage cavity according to Goette (see, however, page 97), when gastrulation is complete (Figure 32c). In this manner, the white yolk reaches the interior during gastrulation, and at the same time the contracting of the blastopore lips overgrows it through epiboly.

Figure 33a shows a beginning gastrula of a rotated embryo, in which a large amount of white yolk still reaches to the vegetal surface. With

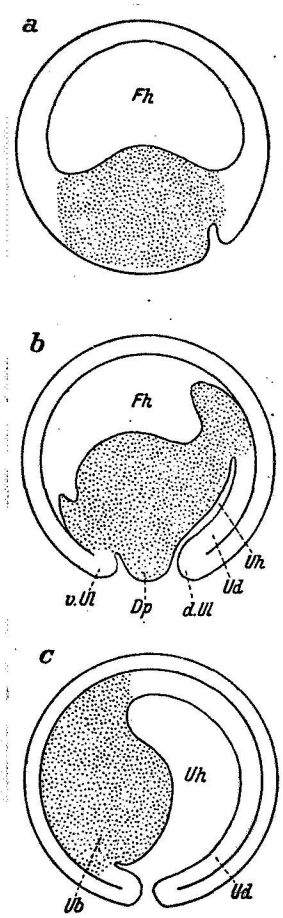


Figure 32. Three stages of normal gastrulation shown in a median section according to Goette. White yolk, stippled; Fh, cleavage cavity; Uh, primitive gut cavity; Dp, yolk plug; d.Ul, dorsal blastopore lip; v.Ul, ventral blastopore lip; Ub, floor of primitive gut; Ud, roof of primitive gut

reference to Figures 33a and 38 it should be noted that the vegetal side is downward, which is not usual in the embryos treated in this study. A blastopore groove (d.Ri in Figure 33a) forms on the dorsal edge of the very white material (white yolk) which reaches to the vegetal surface. In Part III, Section 1, we determined that a rotated embryo may have one or many cleavage cavities, and that their locations vary considerably. We have simplified all the conditions having to do with cleavage cavities by showing only one in our schematic; this is located in the center of the white yolk.

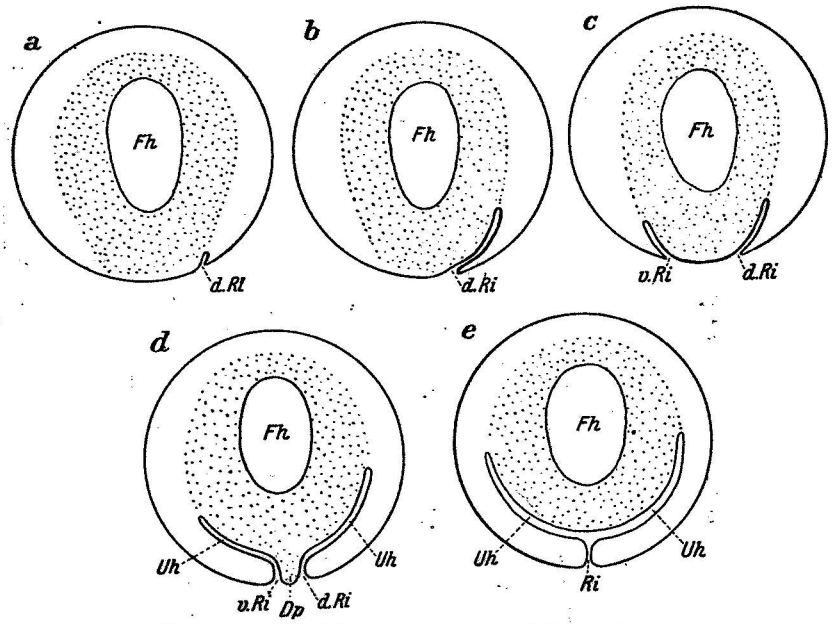


Figure 33. Schematic sectional sketches illustrating gastrulation with the formation of a yolk plug. Discussion in the text. d.Ri, dorsal groove; v.Ri, ventral groove; Ri, secondary groove; all other symbols are the same as in Figure 32

If material tucks over the blastopore lip located dorsal from the very white material (Plate X, Figure 46), conditions as shown in Figure 33b result. A primitive gut roof is formed dorsally. The gastrulation cavity separates the white yolk from the surface material and does not /426
force it into the cleavage cavity. This condition is, however, always only transitory, since a blastopore groove, which was at first purely dorsal, now extends itself around the entire white complex or is completed by the addition of a second blastopore groove. If this occurs, tucking also takes place around the ventral blastopore groove, but as in all the cases reviewed only minimally as in normal embryos (Figure 33c). The ventral blastopore groove also penetrates only between the white yolk and the surface material and does not force the yolk into the cleavage cavity.

Figure 33c shows a section through both a "seemingly normal roundish blastopore" and an "initially wide blastopore groove." In both cases, the very white material, which initially reached up to the vegetal field, is submerged and epibolically overgrown by the blastopore lips, as is shown in Figure 33d. The completed process is shown in Figure 33e. If the very white complex has initially rather small dimensions, the

conditions at the beginning of the process are shown in Figure 33d, and not 33c. After the submergence and epibolic overgrowing of the yolk plug is completed, there is then a slit in the vegetal side, which extends into the depth to the white yolk matter and forks at that point (Figure 33e). In this case, two usually slit-shaped gastrulation cavities are present, as well as two primitive gut roofs, one dorsal and one ventral. The gastrulation cavities as well as the primitive gut roofs are opposedly oriented. /427

According to this schematic comparison between normal and modified gastrulation, the following significant differences exist between them. First of all, the white yolk of a normal embryo submerges into the interior during gastrulation and thereby completely fills the cleavage cavity, or at least it fills most of it. In the rotated embryo, the white yolk, for the most part, penetrates into the interior of the embryo before gastrulation, and the remainder, which is only submerged during gastrulation, is not pushed into the cleavage cavity. Secondly, a primitive gut roof forms only dorsally in a normal embryo, while it forms both dorsally and ventrally in the rotated embryo. One might therefore state that the difference between a normal and a rotated embryo is only that the tucking around the ventral blastopore lip is much greater in the later. One must also add that, depending on the direction of the wide groove in the rotated embryo, a primitive gut roof may be formed toward the right and left, right-dorsal and left-ventral, etc. They are obviously always, or at least usually, opposedly oriented. This also appears to be true when the blastopore is of the "seemingly normal, roundish" variety. Proof of this will be furnished in Part V. A third difference between the normal and the rotated embryo seems to be that the primitive gut roofs are frequently, wholly or in part less sharply defined from the surface material lying atop them. One is therefore not always able to distinguish between ectoderm and primitive gut roof, and between tucked material and material which initially (i.e., at the beginning of gastrulation) was already in place. We shall return to this later.

b. Gastrulation without the formation of a yolk plug

Gastrulation without the formation of a yolk plug begins, according to surface appearance, with the formation of a thin pigment line which deepens into a likewise thin slit: the "initially small groove." The surface aspect also indicates that material must tuck around both edges of this narrow slit into the interior. The edges of such a narrow slit almost invariably enclose a yolk plug in one or two places. As we mentioned above (page 110), such locations where this type of yolk plug is found within the narrow slit will not be taken into consideration here. /428

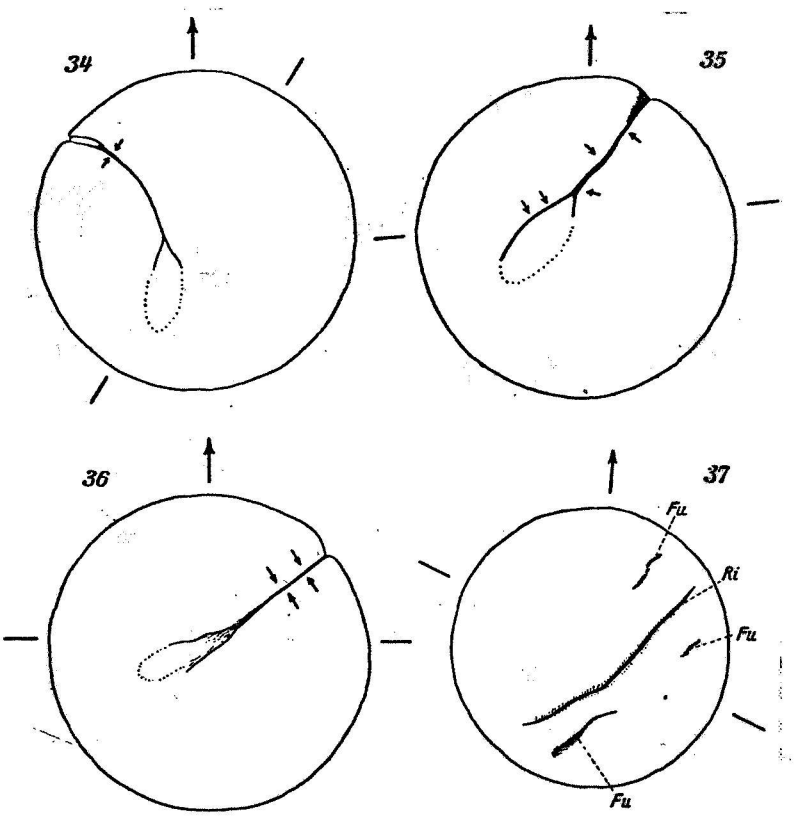


Figure 34. Embryo VII 2, vegetal side; surface view of section, Plate X, Figure 47.
Figure 35. Embryo VII 3, vegetal side; surface view of section, Plate X, Figure 48.
Figure 36. Embryo VII 4, vegetal side; surface view of section, Plate XI, Figure 49.
Figure 37. Embryo IX 16, vegetal side; surface view of section, Plate XI, Figure 50.
Fu, furrows; Ri, blastopore groove

To illustrate the internal processes in this type of gastrulation, we are presenting sections taken from four embryos shown in Figures 34-37 in the condition at the time of fixation. All four embryos have a narrow blastopore groove. In Figures 35 and 36 it encloses a yolk plug at one end. In Figure 34 it encloses such a plug on both ends. The tucking-in, the occurrence of which is evident in the superficial aspect, is indicated in Figures 34 and 36 by arrows. All these embryos were cut in such a manner that the sections in the middle area of the groove run through the groove itself almost perpendicularly. One section from each series showing this middle section, where the groove first appeared, is presented.

In Embryo VII 2 (Plate X, Figure 47, from which Figure 34 was taken), the groove has already cut rather deep into the interior dorsal from the white yolk and lateral from the cleavage cavity. Dorsal from this slit, one may clearly see that a good portion of the primitive gut roof has been tucked into the interior. Judging from the surface appearance, one had the impression shortly before fixation that material was tucking around both edges of the groove into the interior. According to the section series, however, significant tucking occurred only over the dorsal edge. The tucking around the ventral edge was slight in comparison. The primitive gut slit is bounded in the dorsal direction by small, plasma-rich cells which are strongly pigmented on their free surface. In the ventral direction, on the other hand, it is surrounded by quite typical white yolk cells which are pigmented faintly, or not at all, on the free surface, and which seem to be in the interior at the onset of gastrulation. Only in the vicinity of the place where the primitive gut opens to the outside are the cells which are ventral to it also somewhat richer in plasma. It seems, therefore, that only a slight tucking occurred ventrally. For the same reasons, it may be assumed that the blastopore slit enclosed the yolk dorsally and, at the most, only thrust slight amounts of it ahead into the cleavage cavity.

In Embryo VII 3 (Plate X, Figure 48, from which Figure 35 was taken), the primitive gut slit has not penetrated as deeply into the interior as in the previous example. It does not lie to one side of the white yolk, but rather is directed toward its middle portion. At its inner end, in the depths, it forks in two opposite directions, somewhat to the right and left, and corresponding therefore to the orientation of the groove on the surface. Both branches of the fork are still very short, with one being slightly longer than the other. It is worth mentioning that the forking of the slit is generally confined to that region of the embryo where the groove first appeared. /429

The cells to the right and left of the primitive gut slit, which is recognizable along the major part of its course only as a pigment line, do contain white yolk, but they also contain much plasma and pigment and are rather small. Their location in the interior of the embryo may only be understood when one assumes that they represent surface material which has tucked into the depths.

In Embryo VII 4 (Plate IX, Figure 49, from which Figure 36 was taken), the groove leads to a rather markedly forked primitive gut on the inner end, in the area where it first appeared. One branch of the fork is much longer than the other. In the outward direction both branches of the slit are bounded by a pigmented epithelial cell complex, and may both be designated as primitive gut roofs. The primitive gut slit, which at least in this embryo shows a certain widening of its lumen, is bordered by true white yolk cells toward the inside; i.e., the direction where the primitive gut floor should be found. These white /430

yolk cells have no pigment and are loosely associated. We see, therefore, that two primitive guts, which split apart in two opposite directions in the interior of the embryo, are being formed. They have a common opening to the outside in the groove. Each is covered by a primitive gut roof, which was certainly formed by surface material tucking-in over the edges of the groove. One is much longer than the other and both of them are thrust between the dark surface material and the white yolk in the interior or in the cleavage cavity.

Embryo IX 16 (Plate XI, Figure 50, from which Figure 37 was taken) was not fixed until about ten hours after the beginning of gastrulation. For this reason, the primitive gut in the interior has forked earlier than those observed in the previous examples. There can be no question about the fact that each of the two branches of the primitive gut are covered by a complex of material, which has been tucked over the edges of the slit, and which may be designated as the primitive gut roof. One of these in this case is much thicker than the other. Both branches of the primitive gut push, from the base of the groove between the white yolk or cleavage cavity and the primitive gut roof, in opposing directions into the interior of the embryo so that they enclose the yolk.

Summary. In gastrulation without the formation of a yolk plug, the white yolk has already sunken almost completely into the interior during cleavage. Large complexes of very white material do not remain behind on the vegetal surface (where this has occurred, a yolk plug is formed, and such places are not taken into consideration here). As a result of this, the partial process of gastrulation (that is, the submergence of white yolk during gastrulation) is missing, and therewith also the epibolic overgrowing over the yolk. Only the tucking of surface material over the groove edges and the enlargement of the embryo surface, making this tucking possible, remain. This tucking leads to the formation of an initially narrow blastopore groove which at first pushes rather radially as a simple slit into the interior of the embryo. We have in this case, therefore, primarily a single primitive gut (Figure 38a). /431

One may assume that the further course of gastrulation is purely mechanical (in the narrow sense of the word). In the white yolk material in the interior and also apparently in the inside of the cleavage cavities, resistances operating in various ways in individual embryos prevail, which affect the penetration of the direction-indicating primitive gut slit.

When surface material tucks relatively uniformly from both sides of a groove, a single gap is formed, which, as we actually determined, may at first cut almost perpendicularly into the interior (Figure 38a). This proceeds only for a certain distance without disruption, until the internal resistances limit the perpendicular penetration. The primitive gut slit then evades the barrier thus formed. This may occur in one of two

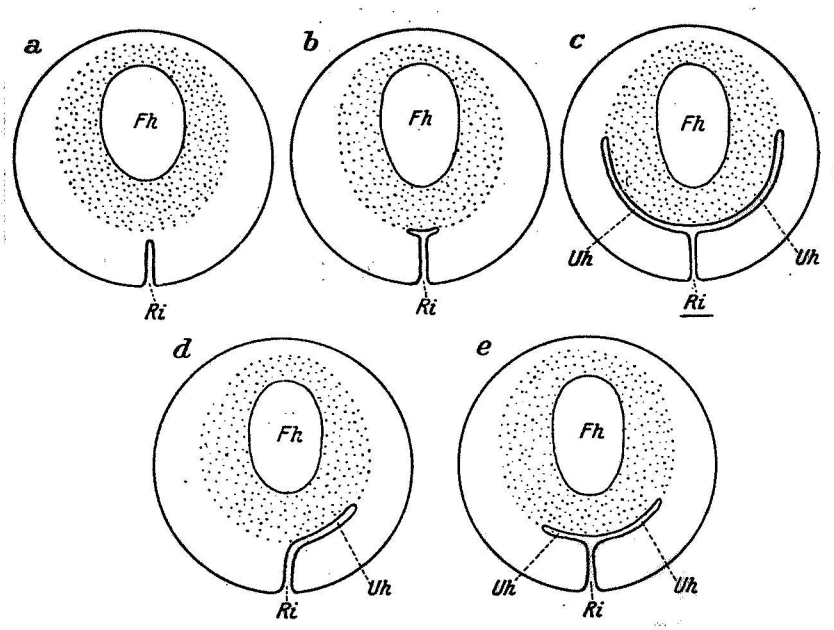


Figure 38. Sectional schematic illustrating gastrulation without the formation of a yolk plug. Discussion in the text. Ri, primary groove; all other symbols are the same as in Figures 32 and 33

ways. First, the slit may fork into the interior toward two opposite sides more or less perpendicular to the direction of the outer groove (Figure 38b). From the forking point, a primitive gut (more or less similar to the movement from the dorsal lip in a normal gastrulation, but here in two opposite directions) pushes itself between the surface layer and the interior white yolk. We see, therefore, that the white yolk material is enclosed on two sides, just as was the case in the above described gastrulation with the formation of a yolk plug (Figure 38c). /432

The initially simple gastrulation slit in the interior may also be affected by the interior resistances in another manner; namely, in that it evades the white yolk on one side and then penetrates further into the interior as an unforked primitive gut (Figure 38d and Plate X, Figure 47). This behavior seems to occur if material tucks exclusively or principally around one edge of the groove. It is also possible that some significance might be attached to the manner in which the originally simple slit penetrates into the interior (more or less perpendicular or initially more oblique) so that it might thrust immediately between the white yolk and the dark upper surface material. Even in those cases where material tucks around only one groove lip at first and where,

as a result of this, a simple, rather long blastopore slit is formed on one side of the white yolk, it seems that a forked gut may be formed afterwards, or as it were, late. This occurs when an ever increasing amount of material begins to tuck around the second lip of the groove, and this tucked over material then pushes between the white yolk and the outer layer on the other side in the same manner as the original slit.

In this last instance, the lengths of the two primitive gut branches would probably always have to be different (Figure 38e). The internal conditions present, for example in Embryo VII 4, help to confirm such an assumption. In this embryo, the forking process begins generally only in the area of the groove, as may be seen in Figure 49 (Plate XI). Otherwise, the primitive gut is only a simple slit which already cuts rather deeply, and whose position corresponds to that of the longer slit in the area of the forking. This determination, together with the fact that one of the slit branches is rather short, indicates that material initially tucked around one lip of the groove on this embryo, obliquely past the white yolk material. The tucking process only began afterwards on the other side.

We question the purely mechanical explanation (mechanical in the restricted sense of the word) for the forking of the primitive gut. The underlying cause of the process, whereby the surface material is tucked over in such a manner that it extends beneath the layer remaining on the surface, is surely more complicated. /433

c. The formation of furrows

In Part IV, Section A.6 we explained that, according to the surface appearance, other furrows are formed during gastrulation in addition to the blastopore grooves. The external aspect of these furrows is exactly the same as that of gastrulation slits. These furrows are, as is shown in the section examination, not without interest.

Plate XI, Figure 51 shows a latitudinal section from such an embryo. Figure 39 shows the superficial aspect of the vegetal surface at the moment of fixation. Aside from the groove on the dorsal edge, three faint furrows may be recognized on this embryo. In the section, they appear as shallow indentations of the superficial cell strata. In his Figure 13, Plate XXXIV, Wetzel (1895) shows the same "cracking" of the surface in the vicinity of the blastopore slit as is seen on the right of the slit in our Figure 39. He surmises that it was caused by an obstacle to invagination. According to this concept, the surface material, in proportion to its dimensions, would not have penetrated into the interior around the blastopore lip to a sufficient degree. It would have piled up immediately in front of the blastopore slit and caused the formation of furrows and folds. (Incidentally, it should

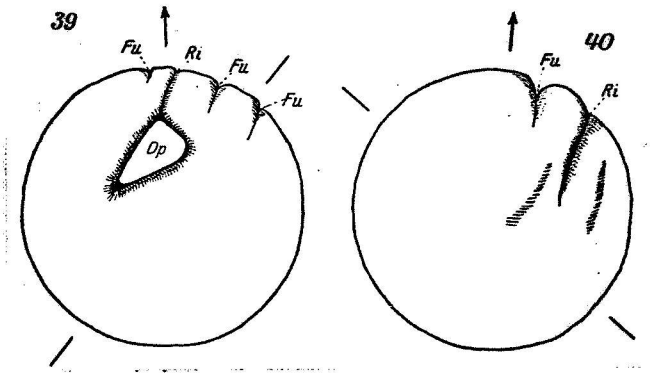


Figure 39. Embryo X 22, vegetal side; surface view of the section shown in Plate XI, Figure 51. Dp, yolk plug; Fu, furrow; Ri, blastopore groove.
Figure 40. Embryo XI 7, vegetal side; surface view of the section shown in Plate XI, Figure 52

also be noted that the narrow blastopore slit is rather perpendicular in the direction of the interior and forks over the white yolk there in two branches, both of which are covered by a primitive gut roof.)

In other instances, these furrows next to the gastrulation groove /434
cut much deeper into the interior of the embryo. This occurred principally in later stages of gastrulation, and they not only give the impression on the surface of being blastopore grooves, but, in section, they also appear as blastopore slits in an early stage of gastrulation. Figure 40 shows a vegetal view of such an embryo about 20 hours after the beginning of gastrulation. There is a rather long blastopore groove curving to the right around the dorsal edge, and to the left of it a deep furrow, which has exactly the same appearance.

In the latitudinal section series, from which Figure 52 (Plate XI) shows an individual slit, it is clear that the slit designated in Figure 40 as Ri is actually the blastopore groove. It penetrates rather radially with a narrow lumen into the interior, but not very deeply, and forks over the white yolk, as was described above in detail for several other embryos. It will therefore be necessary here simply to point out that both primitive gut roofs have penetrated very deeply between the white yolk and the surface material, with respect to the already rather far advanced stage of gastrulation. The depression (Kf) visible in the section on the left ventral side is possibly already to be considered the bounding of a head anlage. The two small cleavage cavities present in this embryo give the impression that they were, by way of exception, both displaced by, and filled with, the white yolk.

Accordingly, one can, again as an exception, determine that both fork branches of the primitive gut on this embryo are much more spacious than normally would be the case. The slit visible on the surface, which is designated as Fu in Figure 40, has the perfect appearance of a beginning blastopore groove which, at this point, cuts only shallowly into the interior.

In a third case, this furrow formation is even more noticeable. According to the even further advanced stage of development in this embryo, the furrow formed next to the groove reaches even further into the interior. Figure 41 shows the vegetal surface of the embryo at a time shortly before fixation. The blastopore has already closed in the ventral region to a short slit (Um). Dorsal from this blastopore remnant, a wide, deep furrow stretches in a direction oblique to the median.

The embryo was cut into sections running approximately in the sagittal direction. Plate XI, Figure 53a shows a section running through the area of the blastopore slit. Figure 53b shows a section lying to the right or left of the one shown in Figure 53a. The blastopore slit has also penetrated here radially in this instance and in the depths it has split into two forks, one longer and one much shorter than the other, over the white yolk. Most important, the secondary furrow looks exactly like a blastopore slit in the interior of the embryo. In this instance, /435 the end of the furrow deep in the interior even has a forking similar to the structure determined to be typical for the true blastopore groove of the embryo (Plate XI, Figure 53b).

In an attempt to interpret the formation of such furrows, the conclusion is inevitable that a significant increase in the surface occurs in these embryos, which is only partially compensated for by the tucking

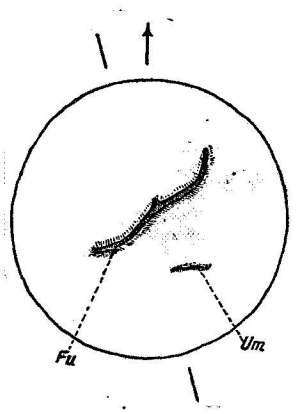


Figure 41. Embryo IX 17, vegetal side; surface view of the section shown in Plate XI, Figure 53, a and b. Fu, furrow; Um, remnant of the blastopore groove

of material around the true blastopore lips. This material, which spreads itself out superficially, has a definite tendency to penetrate into the interior of the embryo. As a result, widely varying attempts to form blastopores take place outside the blastopore region proper, if this region is unable to swallow all this material formed in the growth process. According to this, all these furrows are nothing more than the evidence of falsely channeled growth and tucking tendencies on the part of the surface material. Giersberg (1924) found that, after the blastopore lip was destroyed, a new blastopore lip forms not far from the first one. Under the abnormal conditions prevalent in the rotated embryo, a second blastopore lip may be formed next to the first.

d. Unsolved questions

Even though the gastrulation process in rotated embryos has been explained to a large extent, a number of questions relating to its course in more exact detail are still unanswered. One of these questions will be treated in Part IV, Section C, while the others will be discussed now.

The more detailed type and means of formation of the primitive gut roof and the primitive gut lumen

1. During gastrulation without the formation of a yolk plug, a narrow slit, initially penetrating radially into the embryo, is formed by the tucking of material around both edges of the groove into the interior. In the schematic illustration, Figure 42, the surface cellular material tucking into the interior is black, while the white yolk lying in the depths of the embryo is stippled. According to Figure 42a, the groove therefore is formed by invagination of the surface material as a narrow fold into the interior, until it encounters the white yolk. This primary, simple, primitive gut forks somewhat later, as was described above, and the two branches of the fork enclose the white yolk. /436

This could occur in two ways. The simple fold which was formed previously might bulge outward in two opposite directions as shown in Figure 42b. If this is correct, then the whole formation of the primitive gut, together with its two fork branches, is based on a true invagination in which the floor of the two primitive gut branches is not formed from white yolk, but rather from the invaginated material (shown in black on the drawing). The other possibility is that the fork branches are formed in that both walls of the primary simple groove (if it has thrust forward to encounter the white yolk) fold over in two opposite directions and then grow forward between the white yolk and the surface cell stratum, with a free edge (Figure 42c). If this is correct, only the formation of the simple groove is based on an

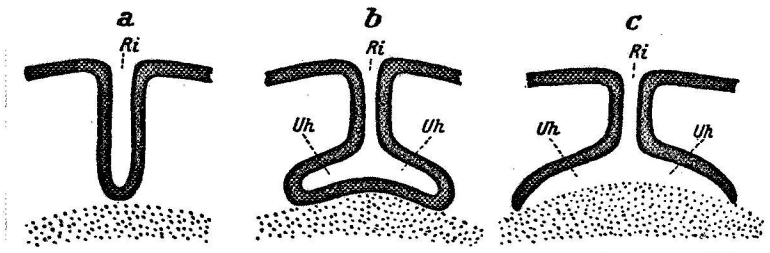


Figure 42. Schematic diagram of the formation of the primitive gut during gastrulation without the formation of a yolk plug. Discussion is in the text. Ri, blastopore groove; Uh, primitive gut cavity

invagination, while the forks of the primitive gut develop from a forward growth of two laminae, and then the floor of the two branches of the primitive gut is formed from the white yolk. No definitive evidence was found that the potential process indicated in Figure 42b ever reaches fruition.

We have already emphasized that the floor of the two primitive gut branches is actually formed from white yolk cells. The second possibility, shown in Figure 42c, is therefore the more probable. It also corresponds to Vogt's ideas concerning the normal formation of the primitive gut roof in Triton (see above). We could not prove that this second possibility actually is in line with the facts. The blind end of each fork branch gives the impression in all of our preparations that the primitive gut roof (black material in the schematic) passes over into the primitive gut floor (white yolk) by folding over, but does not appear simply to lie atop this base with a free edge.

2. The same difficulty exists in gastrulation with the formation of a yolk plug. The beginning of this type of gastrulation is indicated schematically in Figure 43a. The white yolk is stippled, except for the portion of it which reaches to the vegetal surface, which is hatched. The dark material, which abuts the white yolk on the surface, is shown in black. A very shallow blastopore groove formed on both sides of the very white (hatched) material. It deepens, enclosing the yolk plug on both sides. The first beginnings of this type of blastopore groove seem /437 to be formed in the same manner as the primary simple groove in Figure 42a; i.e., by invagination.

The material on the edge of the very white area submerges as a fold into the depths, with both darker and lighter cellular material taking part in this process. In our description of gastrulation according to surface aspects, we demonstrated that white material also seemed

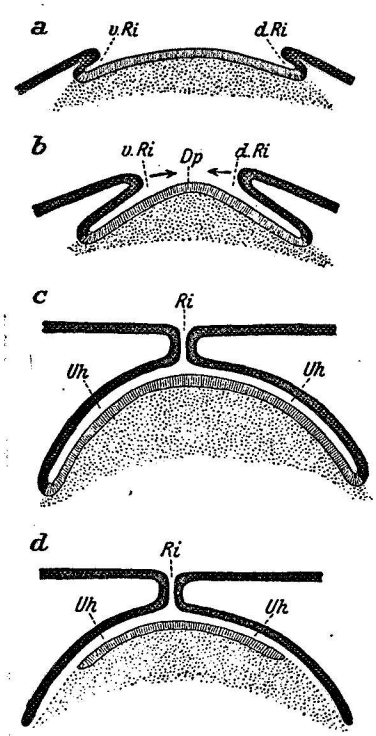


Figure 43. Schematic diagram of a primitive gut formation during gastrulation with the formation of a yolk plug. Discussion is in the text. Symbols are the same as in Figure 33

initially to tuck around such a groove edge. The roof of the two blastopore grooves bounding the yolk plug is formed therefore from dark material (black in the schematic), while the floor is formed from white (hatched in the schematic) material (Figure 43b). The arrows in Figure 43b illustrate how the growing together of the outer edges of the two blastopore grooves effect an epibolic overgrowing of the white yolk. This epiboly is completed in Figure 43, c and d. A secondary unitary blastopore groove is therewith formed from the two originally separate grooves. This groove is then split into two branches over the white yolk in the depths. This epibolic overgrowing of the white yolk occurs in such a manner that surface (black in the schematic) cellular material pushes over it from both sides in folds.

The question now is just how do the fork branches continue to lengthen? It is possible that each of the form-folding branches bulges out even more and continues to enclose the white yolk. In this case, the floor of each branch must be formed entirely of white material

originally located on the surface (hatched in the schematic, Figure 43c). On the other hand, this white surface material might not fold itself any deeper than is shown in Figure 43b. In this case, the primitive gut roof would have to thrust itself between the white yolk and the surface layer of the embryo as a cellular layer with a free edge (Figure 43d). The first possibility is supported in Plate X, Figure 43, about which we stated that here the white yolk seemed to have been pulled into the depths by the forward growth of the primitive gut branches. All the other pictures discussed, however, indicate that the second possibility is the actual one.

3. Finally, the question of just how the primitive gut roof is formed in rotated embryos must remain unexplained to a certain extent. In normal embryos, the entire cell material, which is to become the primitive gut roof and which was previously marginal zone material, is tucked in below the ectoderm. To discuss the question of whether or not this also occurs in rotated embryos, we must first consider in more detail the actual findings concerning the formation and appearance of the two primitive gut roofs than we did previously. /438

In most of the embryos discussed with the aid of section series, we found that, when gastrulation was far enough advanced (when the primitive gut lumen had penetrated sufficiently into the interior), the structure was covered in the outside direction by a rather definitely bounded material complex. This complex may certainly be referred to as the primitive gut roof. Such a roof may be seen in Figure 35 (Plate IX), located dorsal from the blastopore slit. In Plate X, Figure 42a, it is located dorsal and ventral from the yolk plug. In Figure 42b, it is found only ventral from the yolk plug. In Figure 43, it is located both ventral and dorsal from the plug, and only ventral in Figure 44 (Plate X). In Figure 45 (Plate X) and Figures 49-52 (Plate XI), we find a well-defined primitive gut roof over both fork branches of the gut. In Figure 47 (Plate X), a well-defined roof is seen over the unforked primitive gut. Figure 46 (Plate X) shows only a partially well-bounded primitive gut roof. The boundary in Figure 46 is missing in this embryo over the outer portion of the primitive gut and is clearer in the depths. In a very few cases, the primitive gut roof is not bounded at all, as may be seen dorsal from the yolk plug in Figures 41, 42b and 44.

The question arises of how one should consider this mass of different findings: a well-bounded primitive gut roof in the great majority of instances, while in some of the embryos, a material complex of more or less similar disposition and appearance, which was not particularly well separated from the surface material. May one speak of a primitive gut roof at all in these rather rare last cases, from which, among other things, a chorda is formed, or is a corresponding primitive organ absent? We cannot answer this question exactly, either. We should like to assume that the primitive gut lumen is bounded even in these instances

on the outside by a material complex, which functions as a primitive gut roof.

We shall now approach the question of the origin of the various types of primitive gut roofs. The material of the primitive gut roof is at least partially moved into the interior of the embryo through the tucking process in gastrulation. Various sections afford the definite impression that surface material in relatively large thickness has tucked around the blastopore lips into the interior of the embryo (Plate IX, Figure 35; Plate X, Figure 43 (ventral), 44 (ventral), and 47; Plate XI, Figure 49 (above the long branch of the blastopore) and 50 (ventral)). On the other hand, other cases give the impression that only small amounts of surface material have been tucked into the interior, perhaps only the one layer of cells which borders the primitive gut slit on the outside. This layer alone would then have slid from the outside between the white yolk and the dark cell material.

When one compares certain blastula sections (for example, Plate VIII, Figure 25, a-c) or sections from young gastrulae (for example, Plate IX, Figures 29 or 37b) with sections showing late gastrulae in which no clearly bounded roof is present over the lumen of the primitive gut (Plate X, Figures 41, 42b and 44, sometimes dorsal from the yolk plug), one is almost bound to reach this conclusion. It is also possible that, in other cases where a well-bounded primitive gut roof complex is present, the entire thickness of this complex was not tucked over the lips into the interior, but was principally formed by separation (for example, Plate XI, Figures 50 and 51, sometimes to the left in the figure). /439

Finally, it would be possible that, in a few cases, at least that portion of the primitive gut roof farthest from the entrance to the gastrulation cavity is not formed by tucking, but rather by the fact that a surface layer of darker cells split off from the white yolk below it. We did not find any pictures which might confirm this in any of the sections cut through gastrulation stages.

These four different possibilities for the formation of the primitive gut roof in rotated embryos are illustrated schematically in Figure 44. That portion of the primitive gut roof which actually reached the interior by means of the tucking process is cross-hatched. In Figure 44a, a primitive gut roof well separated from the ectoderm is present. This was normally formed solely through the tucking process.

In Figure 44b, only a thin layer has been tucked in and the entire material complex lying above the primitive gut cavity is not recognizably separated into ectoderm and primitive gut roof. In Figure 44c, the conditions are the same with the exception that a slit has been formed in the material not tucked inward, thus separating the ectoderm from the

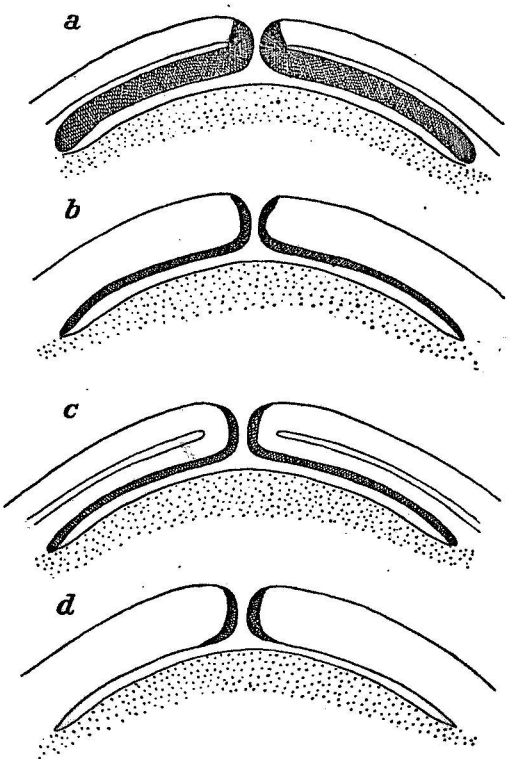


Figure 44. Schematic of the formation of primitive gut roofs in rotated embryos. Explanation is in the text

primitive gut roof. Finally, Figure 44d illustrates the possibility that only very slight tucking inward took place, but that a thick layer of surface cells has raised itself from the white yolk, thereby causing the formation of a primitive gut slit under it. It is possible that, just as in Figure 44c, the ectoderm and primitive gut roof were then also separated through the formation of a slit.

It is clear that we must consider these various methods of primitive gut roof formation, since the findings available to us make it impossible to choose or eliminate one of them or to assume that this formation process in the rotated embryo is unquestionably the same as in a normal embryo. We do believe, however, that this last possibility is true and that to a certain extent (i.e., at least to that shown in Figure 44d), a tucking inward always occurs. For this reason, we feel that it is valid to start with the assumption that the primitive gut roof in rotated embryos always forms only through the tucking inward of surface material. /440

The reason why we cannot decide these questions is clear. Each embryo cut presents to us no more than its condition at the time of fixation. We can never know what has happened in the embryo before or after this time. The fact that the above considerations are not superfluous is based on the fact that, for example, the formation of mesoderm, and therefore of the primitive gut roof, which is not based on the tucking inward of surface material, is attributed to certain anura in normal development.

3. Summary

The beginning of the blastopore occurs in the rotated embryo exactly as in normal gastrulation; i.e., on the surface in the form of a thin, usually branched pigment line. Less often, a pigmented depression is observed. A slit develops from the pigment line, penetrating into the interior of the embryo rather radially, in contrast to the oblique penetration of the normal blastopore slit, until it encounters the mass of white yolk. The place where gastrulation begins is independent of the arrangement of the white yolk in the interior, as well as of the number, locations and sizes of the cleavage cavities.

The longitudinal extension of the blastopore groove, which may be formed either initially as a narrow groove with two folded over edges or only subsequent to the submergence of the very white surface material, is only dependent on the longitudinal extension of white yolk cells on the surface of the embryo. The distribution of material in the interior of the embryo has no influence on this factor.

In terms of inner processes, two major types are distinguishable.

1. In those cases where many white yolk cells are still found on the surface at the beginning of gastrulation, these cells are enclosed in the manner of a normal yolk plug by a more or less oval blastopore and are overgrown epibolically from two opposite sides. One primitive gut lumen forms simultaneously on each of these sides in the interior. These are opposedly oriented and thrust in between the outer darker cells and the interior white cells or the cleavage cavity. A displacement of the cleavage cavity by material pushed into it generally does not take /441
place. As soon as the epiboly is completed, the surface appearance of the blastopore is that of a more or less long groove perpendicular to the direction of the primitive guts. It leads from the outside toward the inside of the embryo in the form of a narrow, simple slit, perpendicularly into the depths, and is transformed into the two primitive gut branches above the submerged white yolk. The gastrulation cavity system consists then as a whole of a simple piece lying on the surface, which forks into two branches over the white yolk in the interior. Primarily and in principal, therefore, these embryos have two separate primitive

guts which are opposedly oriented in the interior. They only become a forked primitive gut secondarily, in that the portions of each of the two primitive guts visible on the surface are joined into a common entrance.

2. The blastopore is formed initially in the form of a narrow groove, over whose two lips surface material tucks over into the interior. This occurs in those embryos, in which almost all of the white yolk has sunken into the interior during cleavage. In these cases also a forked primitive gut is formed, the branches of which extend over the white yolk in opposite directions. In the primary stage in these embryos, only the unforked section of the primitive gut is present. The forked branches are formed secondarily and here also there is no significant invagination of material into the cleavage cavity. In both cases (gastrulation with and without plug formation) the two primitive gut branches are covered toward the outside by complexes of material which may be considered to be the primitive gut roofs. They are certainly formed for the most part during gastrulation by the tucking of material from the surface into the interior. In spite of the fact that several questions have not been explained completely (see previous section), we are proceeding on the assumption that they are formed only in the manner described.

The primitive gut lumina generally do not achieve a large extension up to the end of gastrulation. They are almost always slit-like and generally do not displace the cleavage cavities. As a result of this, and because the white yolk has at least partially sunken into the interior before the beginning of gastrulation and therefore does not need to be completely submerged, the cleavage cavities are usually retained in their entire extension. They may merge with the primitive gut to form a common cavity system. The primitive gut floor is invariably formed, according to the embryos examined on sections by us, from typical white yolk cells which, however, are only present in a thick and compact mass /442
similar to a normal primitive gut floor in the minority of cases. In most instances, the primitive gut lumen and the cleavage cavity are separated only by a few layers of white yolk cells.

Moreover, these yolk cells in question are often arranged so loosely that they can hardly signify a real separation of the two cavities. The cleavage cavities within the white yolk are generally, but not always, bounded by loose, irregularly lying cells which are not arranged in the manner of an epithelium. The white yolk cells bounding the primitive gut lumen form a true epithelial complex except in the rare cases mentioned. Miss Wittmann will describe the very peculiar conditions in the gut lumen of duplications, which follow from this explanation, and which could hardly be understood without it.

C. General Information Concerning Gastrulation of Rotated Embryos and Their Causality

1. Double Gastrulation

As described in the historical part of this study, Wetzel was correct in his opinion that the formation of duplications from rotated embryos is based on the fact that they undergo double gastrulation. Our examinations described in Part IV confirmed the occurrence of such a double gastrulation and added to Wetzel's observations by showing that its course may vary quite widely.

During gastrulation without the formation of a yolk plug, which means the formation of an initially narrow groove, a primitive gut roof overlaying a primitive gut lumen is formed on both sides. This is the kind of double gastrulation described by Wetzel. The same occurs in gastrulation with the formation of a yolk plug and the occurrence of an initially wide groove. A primitive gut roof and a lumen develops on either side, with the only difference being that the two primitive guts come together only subsequent to the time when the initially wide groove has become narrow. They come together through a common slit running in the direction of the surface. If gastrulation begins with the formation of a yolk plug, but leads to the formation of a seemingly normal, roundish blastopore, the further course of events seems to be somewhat different. In many cases, surface material then tucks over two opposite portions of the edge of the blastopore into the interior, so that a primitive gut roof and a lumen also with such a round blastopore are formed on opposite sides and the conditions are exactly the same as in the case of the initially wide groove. /443

It is also very possible that, in the case of the roundish blastopore, the tucking process could occur around the entire edge, but could be much heavier on one side, which would be approximately the same conditions as are present in a normal embryo. This agrees with the fact that embryos do occur, which have seemingly normal roundish blastopores and which resemble normal unitary formations from the beginning (Part V). It is not possible to demonstrate such a normal (simple) gastrulation of a rotated embryo from the beginning through further stages of development with section studies because, even when tucking occurs principally on one side (dorsal), a tucking process may later occur to an even greater degree on the other side, as several described examples indicate, so that two primitive guts are formed.

As we have emphasized so often, the gastrulation process in rotated embryos may vary greatly from case to case. We are principally interested only in those cases in which a double gastrulation occurs in any of the ways described above. Double gastrulation is the rule broken with

few exceptions in rotated embryos, as is confirmed with certainty by our observations of the development of many living embryos. Double gastrulation certainly results, as Wetzel recognized, in the formation of duplications in almost every case, regardless of its subsequent fate. Naturally, one cannot absolutely prove that the duplication formed from rotated embryos is always the result of double gastrulation, but it is highly probable that this is the case.

Since the majority of, if not all, duplications are the result of double gastrulation, the explanation of them requires the uncovering of the causes of double gastrulation.

2. The Untenability of Wetzel's Explanation of Double Gastrulation

Wetzel expressed the following opinion concerning the causes of double gastrulation. 1. The sinking white yolk forms a continuous partition in the interior of the embryo in the direction of the first furrow. 2. As a result of this, as it were, two blastomeres occur. The yolk-containing halves are turned toward each other; the other halves turning away from each other. 3. Due to this, a complete blastula, so to speak, is formed from each of the two primary blastomeres. They are /444
grown together by their vegetal halves and each forms its own cleavage cavity. 4. Since two cleavage cavities are present, an invagination of white yolk takes place in each of them, and thus a double gastrulation occurs. The fact that a simple groove on the surface is formed during this process is secondary and is based only on the fact that surface material on the boundary of the two blastulae is passively drawn into the depths.

This explanation of Wetzel's is, as our detailed results show, untenable in every point. 1. A yolk partition does not form in the interior of the embryo. 2. For this reason, the two "blastomeres" are not constructed in the manner described by Wetzel. 3. As a result of this, the embryo in later stages does not have the organization of a double blastula. This may also be proven by the fact that, as a rule, only one cleavage cavity is present, but two to five such cavities or none at all may occur, and in all these different instances duplications are formed. Since embryos with two cleavage cavities are relatively rare in comparison with all the other possibilities, double gastrulation cannot be based on the presence of two such cavities. 4. Furthermore, the primary beginning of gastrulation begins on the surface, and the primitive gut almost never invaginates into the cleavage cavity.

3. The Actual Bases for the Explanation of Double Gastrulation

According to the state of affairs described above, we must seek the explanation for the occurrence of double gastrulation elsewhere, and

build it on the facts described in Parts II-IV. These facts, and the most important conclusions to be drawn from them, are briefly summarized as follows.

1. In rotated embryos, gastrulation consists of three partial processes, just as in a normal embryo. They are: a, sinking of the white yolk; b, tucking of the surface material; and c, enlargement of the surface of the embryo. The tucking process is the most important with regard to the understanding of double gastrulation.

2. The region of the gray crescent that remains in place in spite of the fact of the yolk rearrangement is capable of performing the tucking process, the dorsal portion intensively so. For this reason, the tucking process usually begins dorsal in the area of the gray crescent. /445
On the other hand, any other place on the surface of the rotated embryo may be capable of tucking in the degree that it is not too loaded with white yolk. It is possible that the groove formation beginning in the region of the gray crescent may be extended in any direction to regions which do not belong to the gray crescent. In such cases, the formation of a groove, and therefore the tucking process, may be said to be dependent on, or induced by, the gray crescent. Secondly, it is entirely possible that the tucking process may occur independently at any point on the embryo, for example on the vegetal field or animal surface. Therefore, any place on the rotated embryo is capable of starting blastopore formation, but, since it most often occurs in the region of the gray crescent, this region must have the greatest capabilities in this respect.

3. In instances where generally complexes of pure white yolk material may be found on the surface of the rotated embryo, the tucking process always begins on the boundary between pure white and darker material. We may conclude from this fact that the tucking of darker or not quite white material is triggered by an influence exerted on it by the pure white material. It is possible that a physiological gradient of some kind exists between cells loaded with white yolk and those without it, and is the triggering factor for tucking on the part of the latter. This is shown best in the formation of an animal blastopore anlage which probably forms only when pure white material has sunken to the animal surface.

4. In a rotated embryo, the arrangement of the yolk matter is changed from the norm, both on the surface and on sections taken from the interior.

The surface aspect never shows the circular white vegetal field. On the contrary, the coloration of the vegetal side may be changed in a wide variety of ways. First of all, random formations consisting of large complexes of pure white material may be visible on the vegetal surface.

In such cases, the blastopore groove is always formed on the edges of these complexes, usually on the dorsal, but sometimes also on the ventral edge (then usually later than the formation of the dorsal groove) and in other locations.

Second, these large complexes of pure white material on the vegetal surface are often extended in the direction of one of the first two furrows (in this case they usually extend onto the animal surface in the form of "band ends") or there are smaller to minimal very white areas scattered in the less white material in the same direction. In this instance, the blastopore groove always forms in the direction in which these pure white areas are extended or distributed, and therefore likewise in the direction of one of the first two furrows. /446

Third, it is possible that no more pure white material may be visible on the vegetal surface. In this case also, a blastopore groove appears, which runs in the direction of one of the first two furrows (possibly even in the direction of the third furrow, but since this occurs only rarely we shall not take it into consideration here).

Fourth, the vegetal surface finally presents, regardless of whether or not pure white places are visible on it, a zone of lighter coloration in every case (light brown or whitish and brown mixed). This lighter zone may either be irregularly roundish or elongated, in which case it constitutes the light or white band. The presence, the size, and the form of the pure white places are independent of the form of the lighter zone. If a light band is developed, they are, however, usually also extended along its longitudinal direction, and are sometimes arranged in rows. In cases where a light band is present, it most usually runs in the direction of one of the first two furrows.

We were able to prove on section pictures that the white yolk in the interior of the embryo does not form a partition in Wetzel's sense, but an irregularly formed mass. For this reason, it is impossible for a certain longitudinal extension of the yolk in the interior to have any influence on the direction of the blastopore anlage. The presence of a light band, the elongated form of a very white complex or the arrangement of such complexes in a straight line cannot, therefore, be determined by the form of the white yolk in the interior. On the other hand, this may not be said of the arrangement of the white yolk located close to the vegetal surface.

5. The blastopore is a "seemingly normal, roundish" one if its edge surrounds a similarly roundish, very white complex. It is an "initially wide groove" if the blastopore lips form on both sides of an elongated very white complex. It is an "initially narrow groove" if it is formed without reference to a visible, very white complex and encloses it, at most, only on one or both of its ends. When a light band is

present and the blastopore is either a wide or narrow groove, this groove always runs in the longitudinal direction of the band. If the embryo has neither a light band nor elongated, very white areas, a /447
forming narrow blastopore groove nevertheless runs in the direction that the band or area would have taken; namely, usually in the direction of one of the two first furrows.

4. Relation of the Facts to Form a Hypothesis Concerning the Causality of Double Gastrulation

The very white areas on the surface determine the direction of the groove. If, on the other hand, no very white areas are visible, the groove is determined by the direction of one of the two first furrows; more specifically, by the direction of the furrow which cuts through the vegetal field during the yolk rearrangement process.

In the rotated embryo, the white yolk must be released from the stationary cortex of the vegetal field before sinking. Extensive experience in the physiology of cell division allows for the assumption that the surface of the egg has a different quality in that meridian through which a furrow is cutting. A furrow, which cuts into the surface of the white field during the loosening of the white yolk from the surface may be so constituted that pieces (preeminently very small pieces) of white yolk cling to it at various places along its length. They are enclosed in the surface cells, so that they may not be recognized but are rather large complexes. If the furrow cuts into the edge of the vegetal field just at the time when the white yolk is detached from the surface, here also complexes may cling and cause places on the rotated embryo, which lie outside the original vegetal field, to appear white (see page 37).

Let us concern ourselves next with those cases in which the smallest pieces of yolk material cling to the furrow and are enclosed in the cells forming there at a subsequent time. In such instances, a streak of cells whose direction coincides with that of the furrow is present in the blastula. These cells enclose white yolk matter, which is not visible. This cell streak, as we know from our observations of white material (see above), affects the dark material abutting it in such a way that it is induced to tuck over. On both sides of this streak, therefore, surface material begins to tuck; i.e., an initially narrow blastopore groove is formed, which runs in the direction of one of the two first furrows.

If larger complexes cling along the furrow cutting in at the time, they are stretched along the furrow or they form a row of white areas /448
in the same direction as the furrow. They cause the formation of a blastopore groove in the direction of the furrow for the same reasons as do the smallest complexes. If these complexes are fairly wide, the blastopore groove must be initially wide.

If the complexes clinging to the furrow during its cutting process are exceptionally large, the blastopore anlage formed cannot be groove-shaped because it must form on the edges of the more roundish white complexes.

The vegetal side of the rotated embryo is, independent of the location and form of the very white areas, still not very white, to a considerable extent. It is, however, whitish to light brown in color in a zone which is irregularly roundish to definitely band-like in shape. We see, therefore, that what we have always designated as a white, roundish zone or a white band is present. According to Wetzel, this band is nothing more than the edge of the white yolk material which is arranged in the form of a partition inside the embryo. If no white band is present, there should, according to Wetzel, be no partition arrangement of the white yolk. We observed, on the contrary, that the white yolk matter is almost never arranged as an inner partition, even if the white band is formed, but that it is present in the form of an irregular, roundish mass on a longitudinal section. This may be explained by the fact that it sinks along all the meridianal planes of cleavage, and not along just one.

However, this must be somewhat different in the direction of the egg axis. The white yolk detaches itself from the vegetal surface (except for pieces which form the very white areas) and then sinks slowly along the meridianal planes of cleavage. At first, it naturally sinks along the first plane of cleavage if the detaching of the white yolk has begun at this stage of development. At first, then, the white yolk does form a partition in the upper region of the vegetal portion of the embryo in the direction of this furrow, as we have emphasized so many times. During the further process of sinking, the second and third meridianal planes cut through, and the white yolk also slides slowly downward along these and acquires thereby an irregular, roundish cross section. As a result of its viscous property, it remains hanging as a layer along those planes of cleavage, along which it has sunken. As a result of this, it does permanently form a sort of partition in the direction of the first furrow in the upper regions, where the sinking process began (but not below, therefore invalidating Wetzel's opinion). /449

If the process takes place as described, this arrangement of the white yolk on the surface would appear as a white band. If the white yolk is more viscous and, therefore, sinks from the vegetal surface more slowly, it is possible that the second furrow will have cut in deeply before much of the white material has moved to any notable distance from the surface. In this case, the yolk will cling along not only one plane of cleavage in the upper portion of the cleaving egg, and the surface will not have a light appearance in one direction only; i.e., it will not present a light band[1].

[1]If the white yolk detaches itself from the surface during the formation of the second furrow, these processes undergo a corresponding shift.

We feel that the occurrence of a white band or an irregular, white, roundish zone can be traced to the degree to which the white yolk has remained in the upper regions, but under the vegetal surface, as a result of its adhesion to the planes of cleavage. This has no influence on the direction and form of the blastopore anlage being formed, since it is independent of the very white material which remains adhered to the upper surface. It also has no influence on the form of the main mass of white yolk in the interior of the egg, which never has the form of a partition. The cause of the varying arrangement of the white yolk in the upper layers of the embryo is its differences in viscosity, and perhaps the varying amount in which it is present and/or the velocity of the cleavage process.

These concepts concerning the relationship between the direction of the first furrows; the formation and direction of a light band or the formation of a light, roundish area; the presence and detailed aspect of very white areas; and the form and direction of the first blastopore anlage are confirmed by all of the cases we observed. The fact that these concepts are rather complicated should not cause any shock, since these intricacies are derived only from the multiplicity of possible yolk arrangements during various stages of cell division, in various stages of cleavage, and with the varying mass and viscosity of the white yolk itself. The above concepts are the only explanations which we have been able to find as yet for the occurrence and various forms of double gastrulation.

We must admit that our earlier work (see Schleip and Penners, 1926) contained a more simple, schematic concept, but a more exact examination of the results led us to a more careful consideration of the exceptional variability of these processes. We also realize that these concepts are /450
still hypothetical. They may, however, be checked experimentally by comparing the surface picture with the stage of cell division present at the beginning, and during the further progress, of the yolk rearrangement. It is also possible to conduct exact morphological examinations of the constitution of the vegetal surface of the blastula, especially with respect to the dispersion of white yolk in the surface cells. Such morphological examinations could furnish further evidence useful in the evaluation of the above concepts.

EXPLANATION OF PLATES VI-XI /452

The dorsal side of the egg is oriented upward in Plates VI and VII. Only in Figure 11 (Plate VII) is this orientation different; in this case it is marked with an arrow. The orientation of the embryo in Figure 14 (Plate VII) is unknown. Dashes alongside the figures indicate the oblique or frontal position of the first furrow. Where these dashes are lacking, the first furrow has a median location.

Abbreviations

a, animal
d, dorsal
Dp, yolk plug
E, depression
Fh, cleavage cavity
Fh^1-Fh^5, cleavage cavities 1-5
Fu, furrow
Kf, cephalic furrow
l, left
r, right
Ri, blastopore groove
S, slit (wound)
Ud, primitive gut roof
Uh, primitive gut cavity
Um, remnant of the groove after closure
v, ventral
veg, vegetal

Plate VI. Photographs of the Surface Aspect of Cleavage and Gastrulation Stages

Figure 1. Embryo 12 (25). a, immediately after pressing and rotation, during the first cleavage; April 2 at 5:00 p.m. b, second cleavage completed; 7:00 p.m. c, third cleavage completed; 9:30 p.m.

Figure 2. Embryo XVI 1. a, immediately after pressing and rotation, first cleavage almost completed; April 6 at 6:10 p.m. b, second cleavage almost completed; 6:42 p.m. c, second cleavage completed; 6:55 p.m. d, third cleavage almost completed; 7:20 p.m. e, 7:40 p.m. f, 8:30 p.m. /453 g, 9:30 p.m. h and h^1, 10:30 p.m. i, April 7 at 9:30 a.m.

Figure 3. Embryo 14 (25). a, immediately after pressing and rotation, first cleavage completed; April 2 at 6:00 p.m. b, 9:15 p.m. c, 11:55 p.m. d, April 3 at 8:30 a.m. e, 3:15 p.m.

Figure 4. Embryo 13 (25). a, blastula; April 3 at 9:35 a.m. b, beginning of gastrulation; 4:20 p.m.

Figure 5. Embryo 16 (25). Blastula

Figure 6. Embryo 15 (25). Blastula

Figure 7. Embryo 17 (25). a, blastula; April 6 at 9:00 a.m. b, gastrula; 5:15 p.m.

Figure 8. Embryo 18 (25). Blastula

Figure 9. Embryo 19 (25). a, blastula; April 3 at 10:00 a.m. b, beginning of gastrulation; 4:00 p.m.

Figure 10. Embryo 20 (25). a, variously advanced stages of gastrulation; April 6 at 10:30 a.m. b, 1:15 p.m. c, 3:15 p.m.

Plate VII. Photographs of the Surface Aspect of Blastula and Gastrula Stages

Figure 11. Embryo 21 (25). a, beginning of gastrulation; April 6 at 11:00 a.m. b, groove; 3:15 p.m.

Figure 12. Embryo XVI 2. Blastula; surface aspect corresponding to section, Plate III, Figure 23.

Figure 13. Embryo 11 (25). a, blastula; April 6 at 9:30 a.m. b, groove with two plugs; 5:25 p.m.

Figure 14. Embryo 22 (25). Groove from beginning to closure with yolk plug formation. a, April 1 at 6:45 p.m. b, 10:30 p.m. c, April 2 at 5:00 a.m. d, 8:30 a.m. The orientation of the groove to the medium plane of the egg is unknown.

Figure 15. Embryo XIII 1. Long groove with a plug at one end. a and a^1, April 4 at 2:30 p.m. b^1, 7:00 p.m.

Figure 16. Embryo XII 1. Gastrulation with formation of a long groove and a furrow

Figure 17. Embryo XIII 3. Gastrulation with the formation of a long groove and with the appearance of furrows and wrinkles. a, April 4 at 10:25 p.m. b, April 5 at 12:10 a.m. c, 2:45 a.m. d, 5:31 p.m. e, April 6 at 9:45 p.m.

Figure 18. Embryo XII 2. Seemingly normal gastrulation. a, April 4 at 3:15 p.m. b, 6:00 p.m. c, April 5 at 2:00 a.m. d, 9:00 a.m.

Figure 19. Embryo XIII 2. Seemingly normal yolk plug with the formation of furrows. a, April 4 at 3:20 p.m. b, April 5 at 2:30 a.m.

Plate VIII. Photographs Made of Sections through Blastulae

Figure 20. Embryo XII 3. Section parallel to a meridian.

Figure 21. Embryo XV 1. Section parallel to a meridian.

Figure 22. Embryo XIV 1. Section parallel to a meridian.

Figure 23. Embryo XVI 2. Section parallel to a meridian, taken from the embryo in Plate VII, Figure 12.

Figure 24. Embryo VII 1. Section parallel to a meridian.

Figure 25, a-c. Embryo XII 4. Section parallel to a meridian. /454

Figure 26, a-c. Embryo VIII 1. Latitudinal section.

Figure 27. Embryo 9 (25). Section parallel to a meridian (cleavage was retarded by cooling).

Plate IX. Photographs of Sections through a Blastula (Figure 28) or through Beginning Gastrulae

Figure 28. Embryo 9 (25). Section parallel to a meridian (cleavage was retarded by cooling).

Figure 29. Parafrontal section from embryo in Figure 14.

Figure 30. Parafrontal section from embryo in Figure 15.

Figure 31. Parafrontal section from embryo in Figure 16.

Figure 32. Section from the embryo in Figure 17, perpendicular to the direction of the blastopore groove.

Figure 33. Section from the embryo in Figure 18, perpendicular through the blastopore groove.

Figure 34. Section from the embryo in Figure 19, perpendicular to the blastopore groove.

Figure 35. Section from the embryo in Figure 20, perpendicular to the blastopore groove.

Figure 36. Section from the embryo in Figure 21, perpendicular to the blastopore groove.

Figure 37, a-c. Sections from the embryo in Figure 23, perpendicular to the longitudinal direction of the blastopore.

Plate X. Photographs of Sections through Early and Intermediate Gastrulae

Figure 38. Section from the embryo in Figure 22, perpendicular to the longitudinal direction of the gastrulation pit.

Figure 39, a and b. Paramedian section from the embryo in Figure 24.

Figure 40. Embryo VIII 4. Horizontal section.

Figure 41. Section from the embryo in Figure 25, perpendicular to the groove.

Figure 42, a and b. Paramedian sections from the embryo in Figure 26.

Figure 43. Embryo IX 18. Section oblique through the yolk plug.

Figure 44. Section from the embryo in Figure 27, perpendicular to the groove.

Figure 45. Section from the embryo in Figure 28, perpendicular to the groove.

Figure 46. Embryo VIII 2. Section perpendicular through the roundish, arcuate blastopore.

Figure 47. Section from the embryo in Figure 31, perpendicular to the groove.

Figure 48. Section from the embryo in Figure 32, perpendicular to the groove.

Plate XI. Photographs of Sections through Later Gastrulae

Figure 49. Section from the embryo in Figure 33, perpendicular to the groove.

Figure 50. Section from the embryo in Figure 34, perpendicular to the groove.

Figure 51. Horizontal section from the embryo in Figure 36.

Figure 52. Horizontal section from the embryo in Figure 37.

Figure 53, a and b. Sections from the embryo in Figure 38, perpendicular to the direction of the furrow.

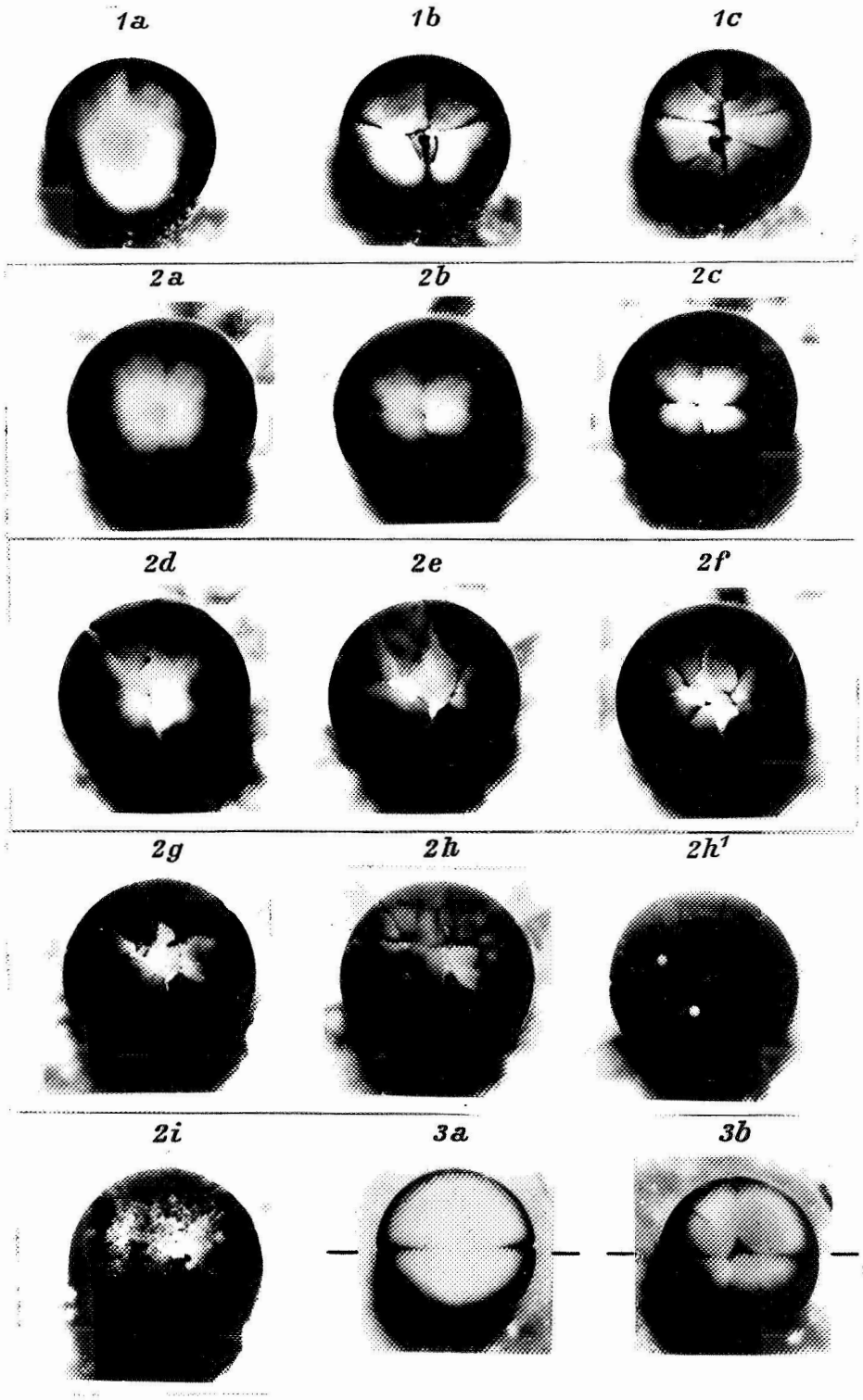
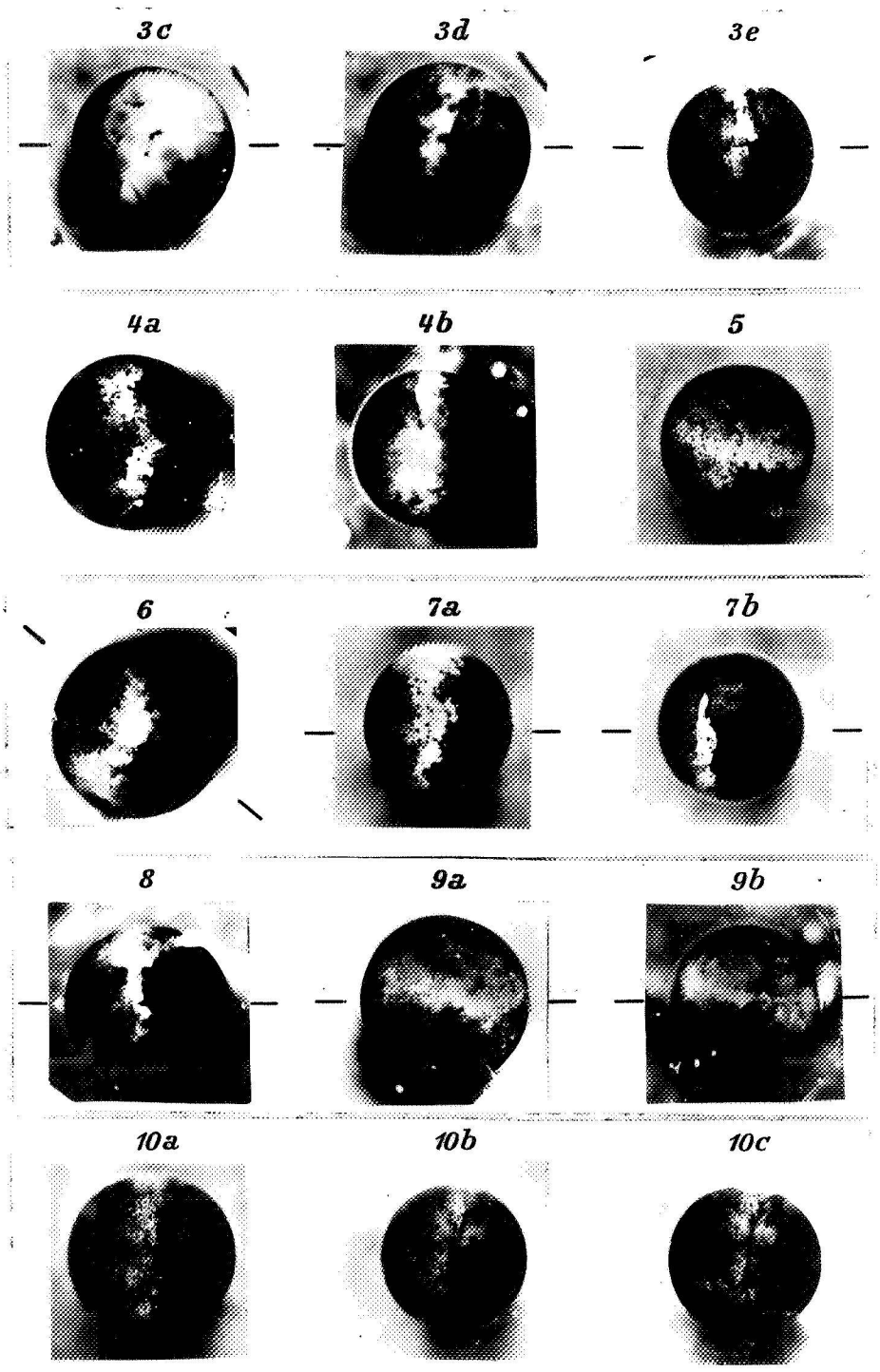


Plate VI



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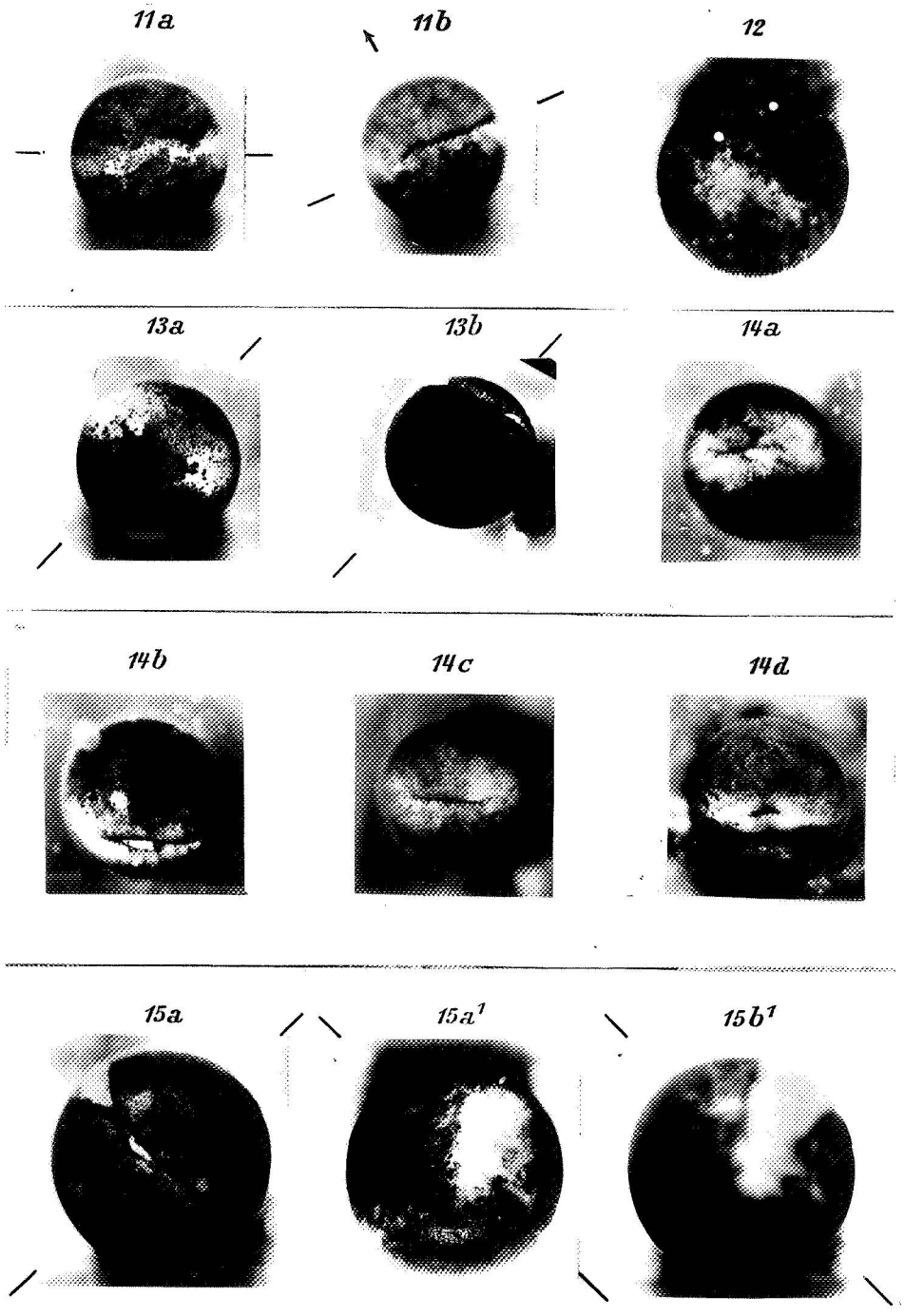
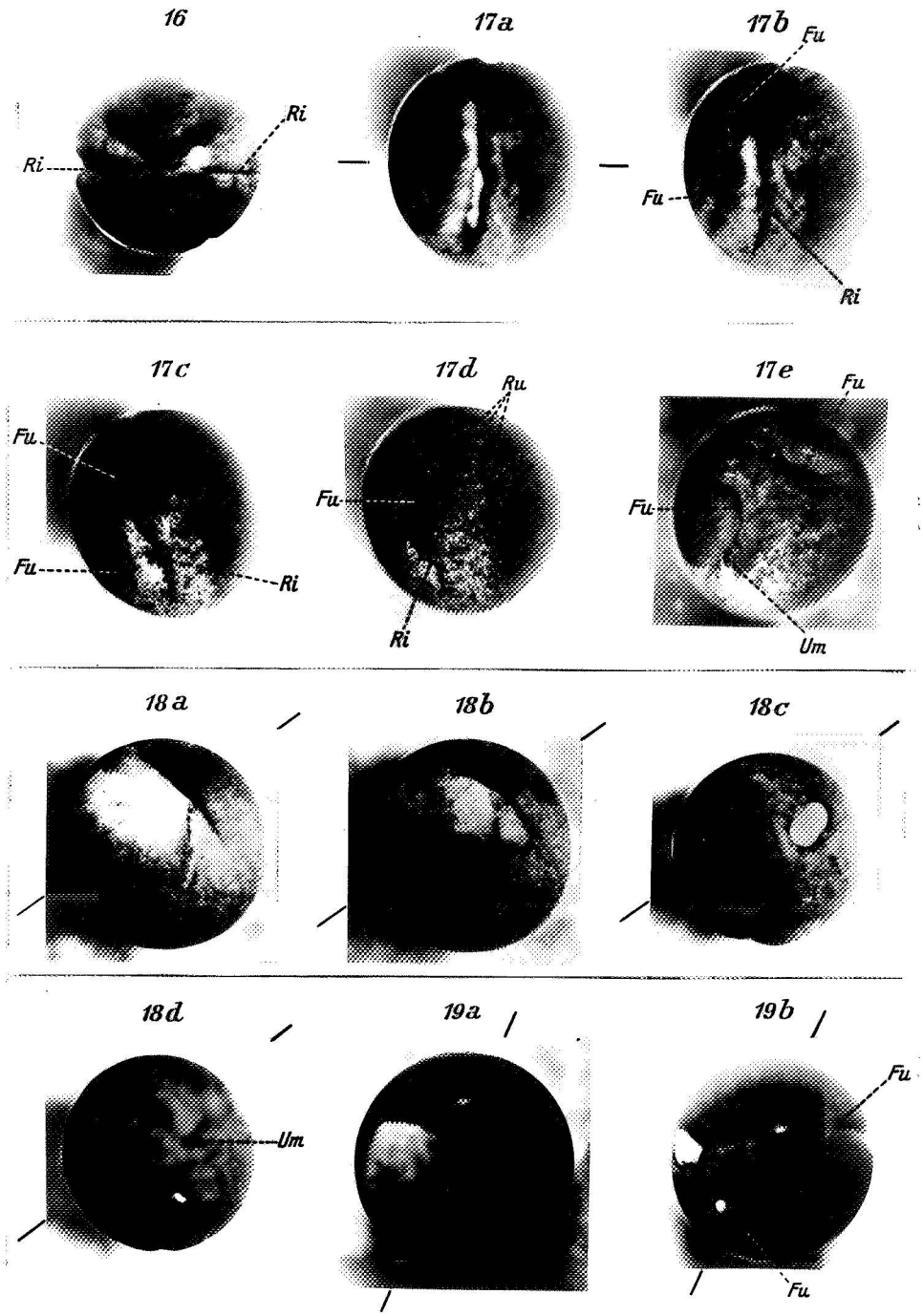


Plate VII



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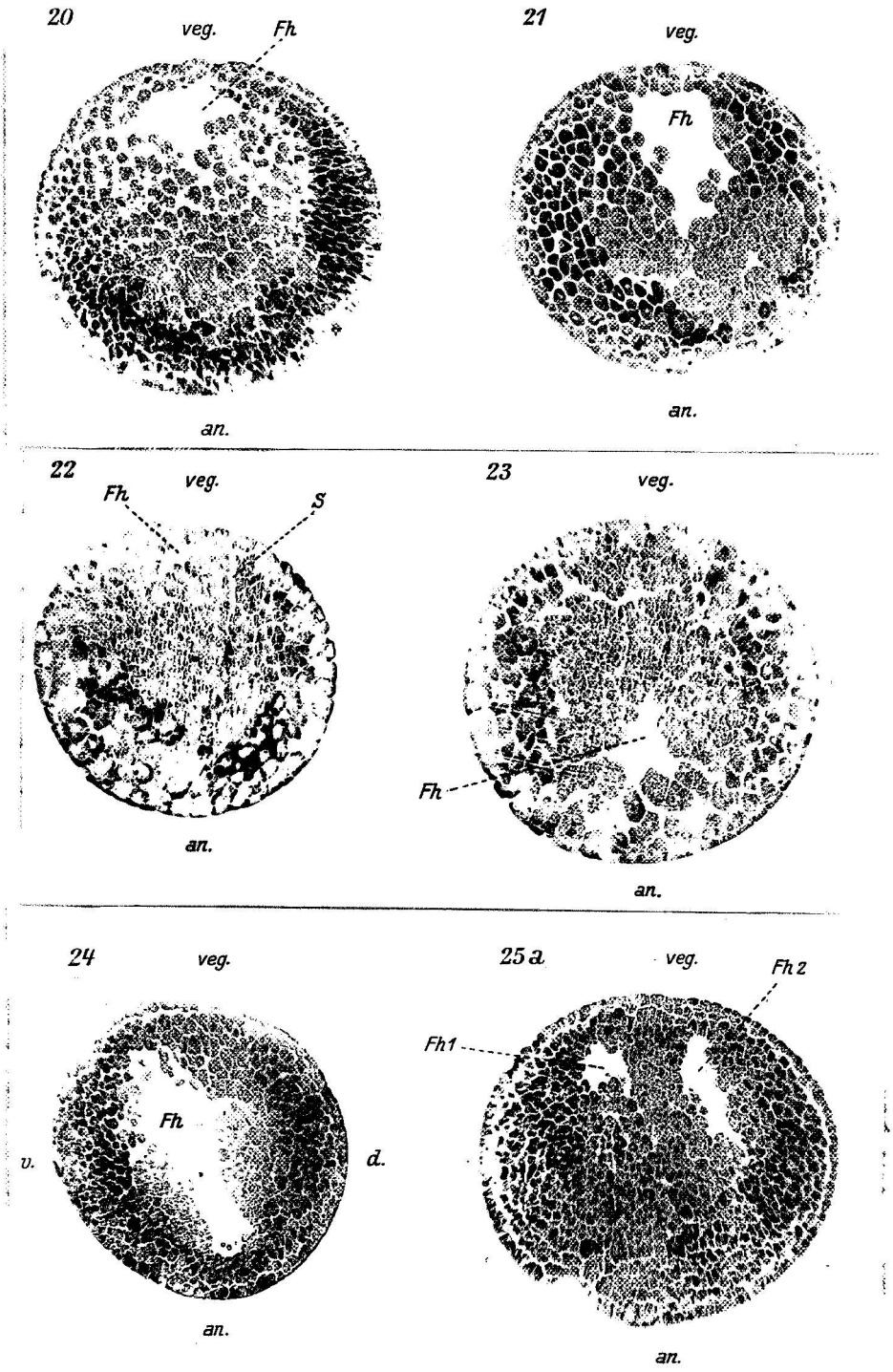
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25 a
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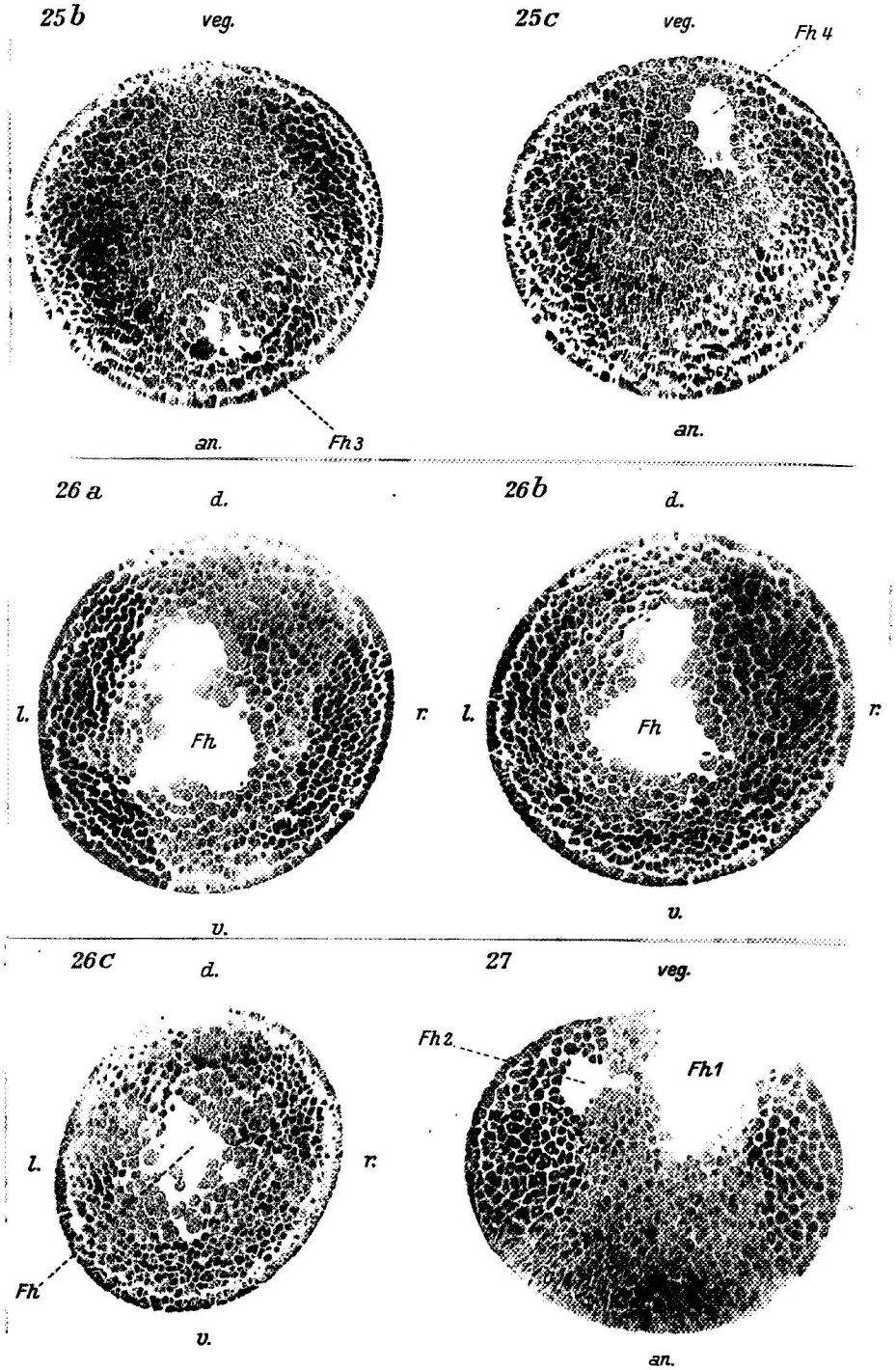
an.

Plate VIII



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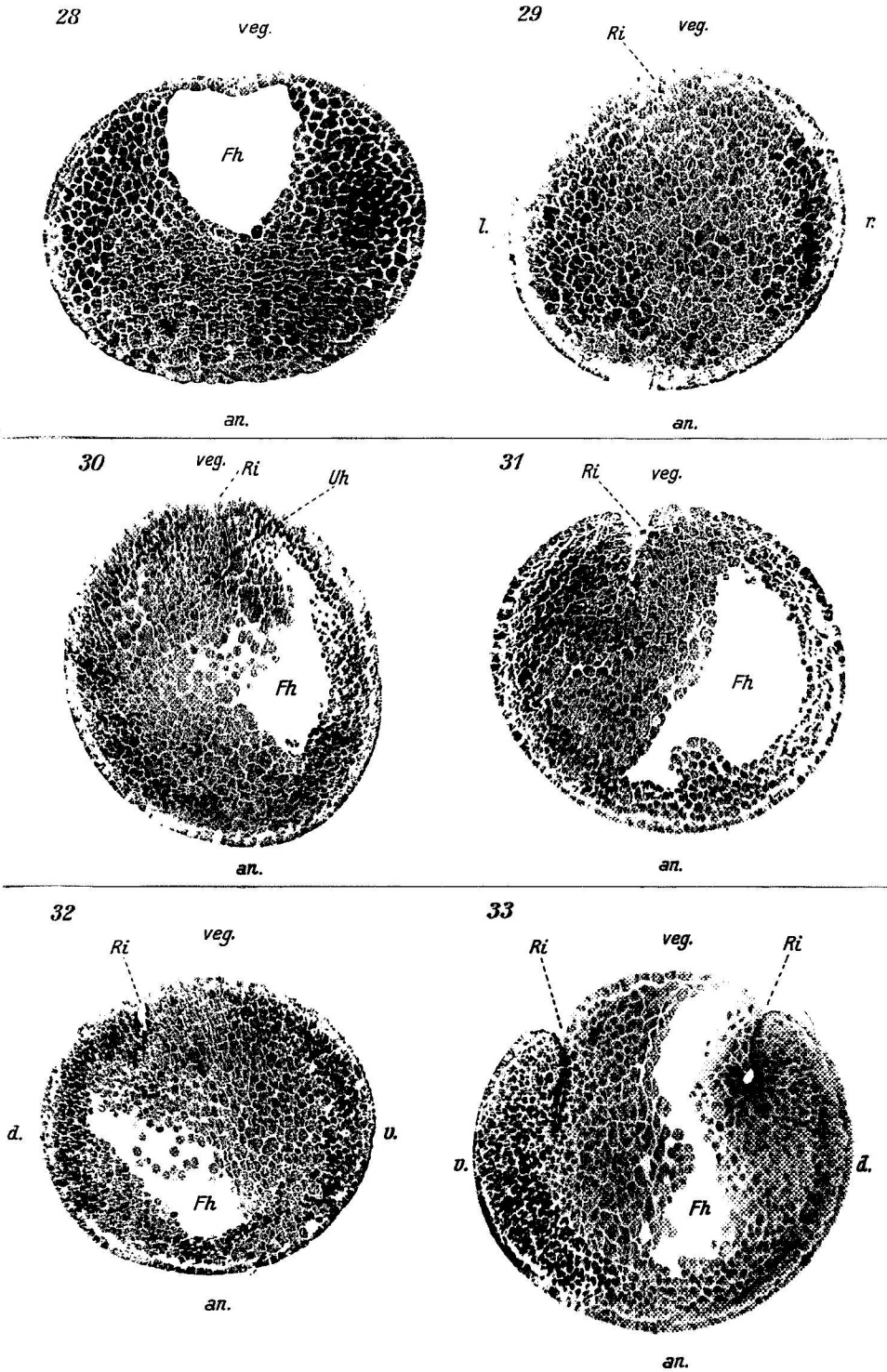
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Plate IX

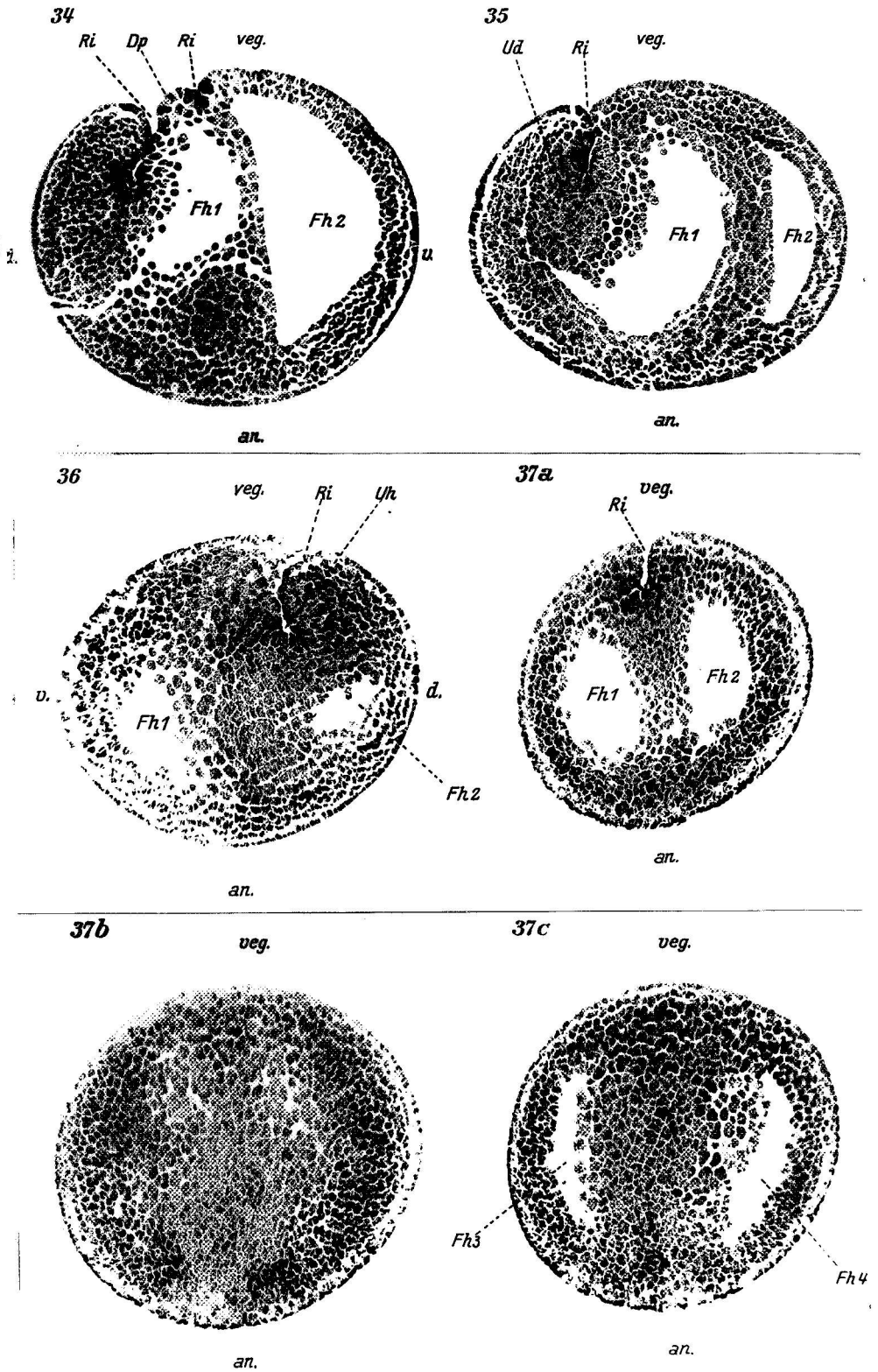
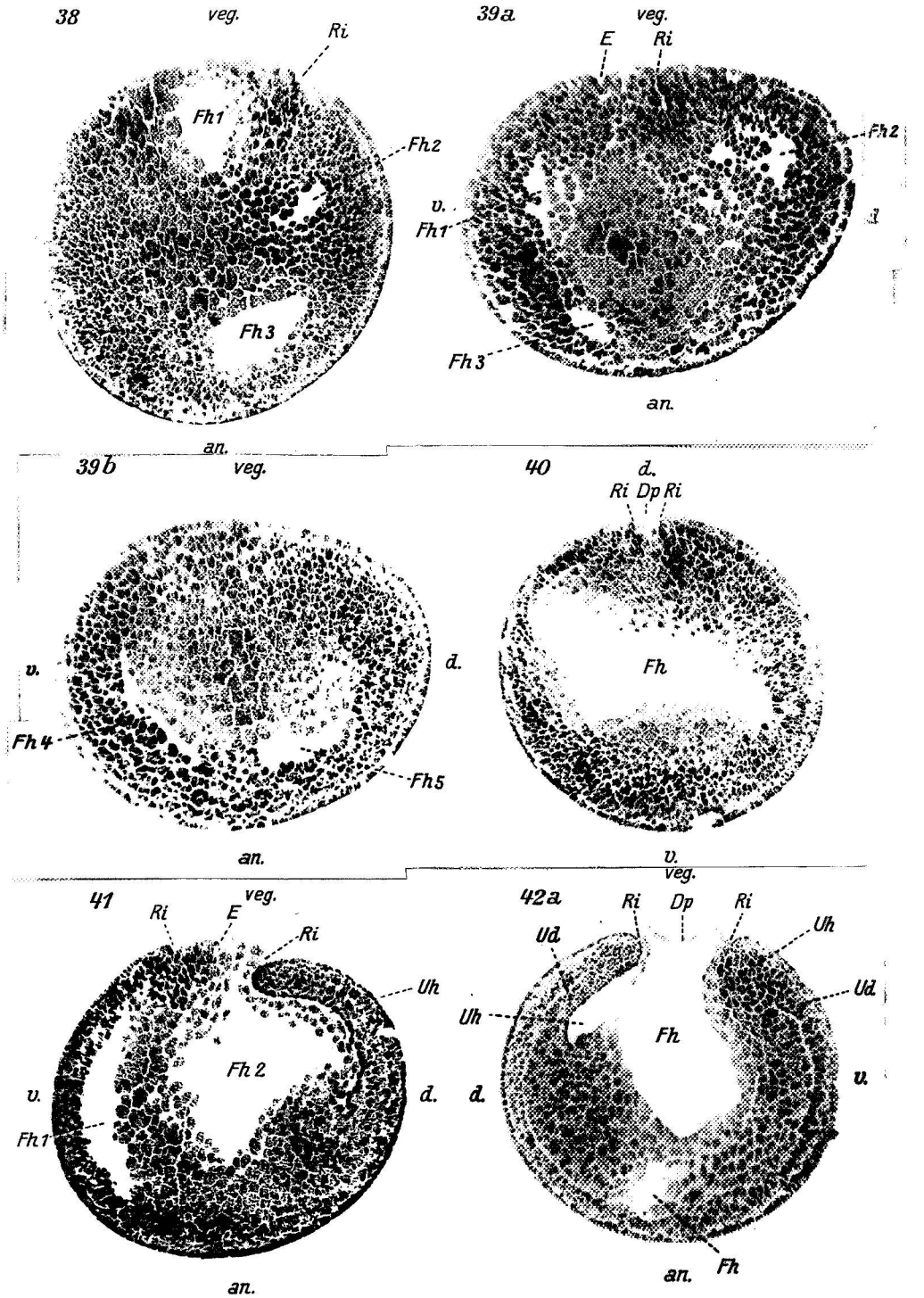


Plate IX (continued)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veg.
v.
d.
Fh4
Fh5
an.
40
d.
Ri Dp Ri
Fh
v.
41
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E
veg.
Ri
Uh
Fh 2
v.
d.
Fh1
an.
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Ud
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Plate X

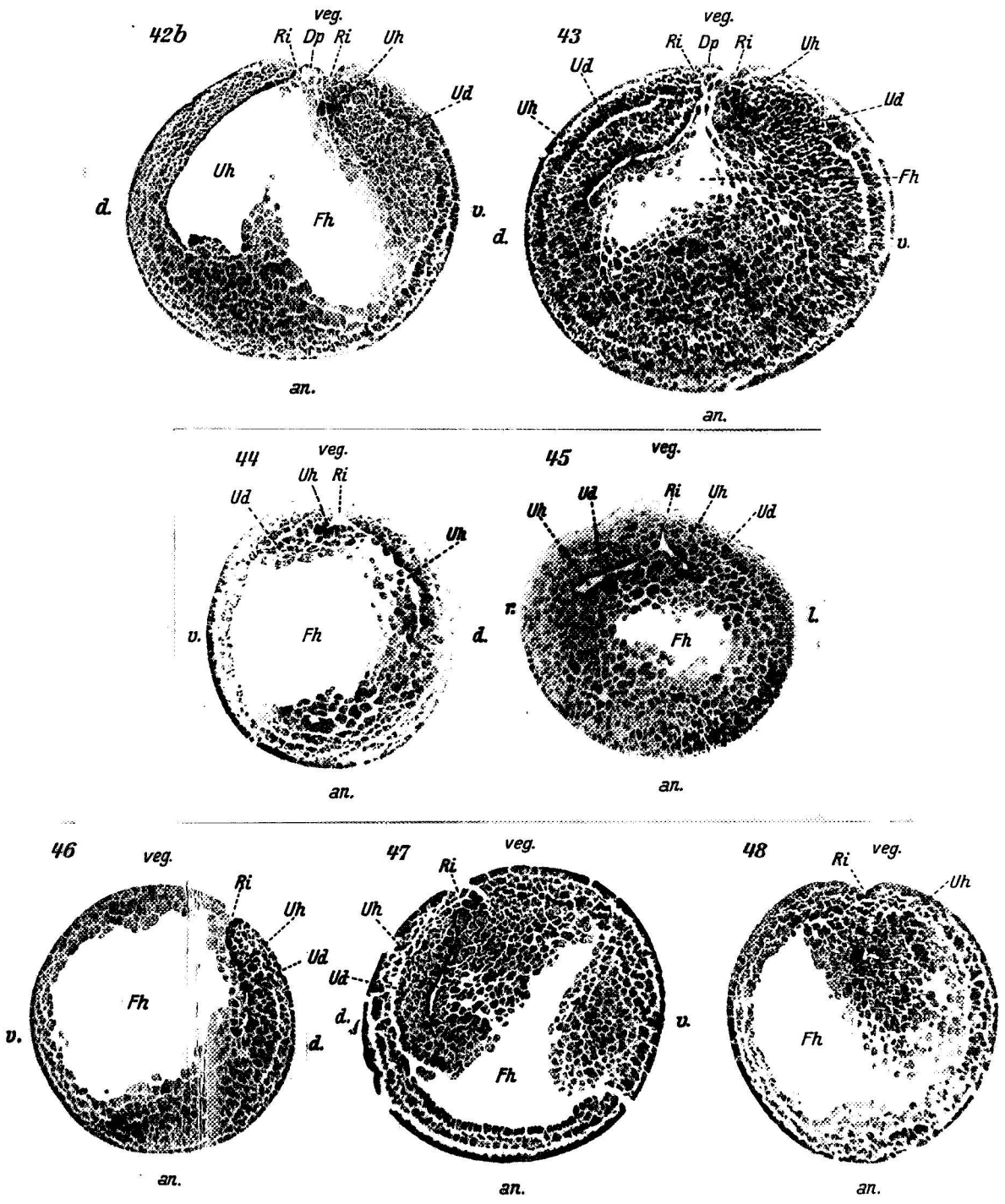
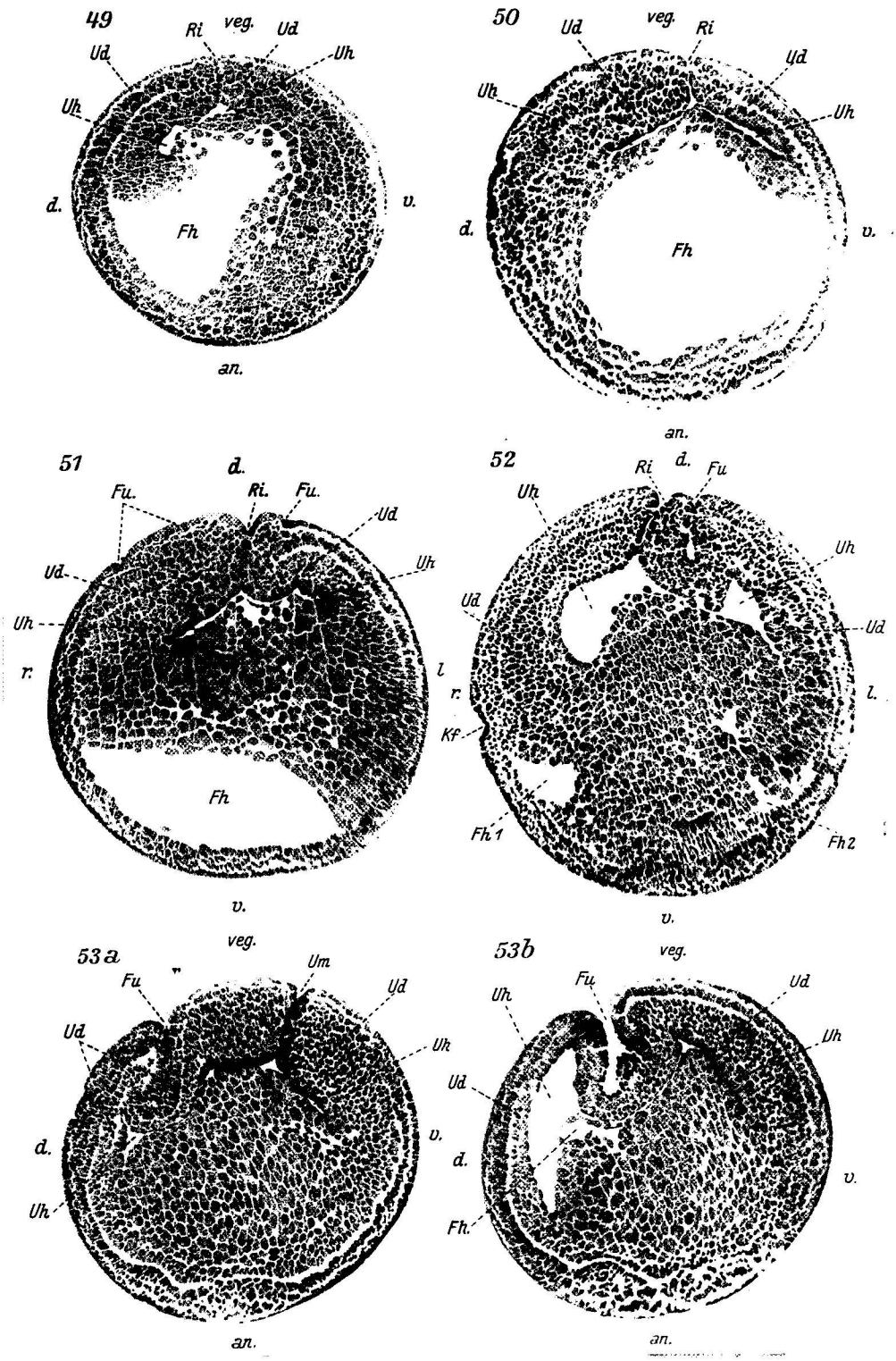


Plate X (continued)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Fh 2
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veg.
Fu
Ud
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Fh.
an.

Plate XI

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